β-galactosidase of ROSA26 mice is a useful marker for detecting the definitive erythropoiesis after stem cell transplantation

Asari, Sadaki

(Degree)
博士（医学）

(Date of Degree)
2004-09-30

(Date of Publication)
2013-04-16

(Resource Type)
doctoral thesis

(Report Number)
甲3179

(URL)
https://hdl.handle.net/20.500.14094/D1003179

β-GALACTOSIDASE OF ROSA26 MICE IS A USEFUL MARKER FOR DETECTING THE DEFINITIVE ERYTHROPOIESIS AFTER STEM CELL TRANSPLANTATION

ROSA26 マウスのβ-ガラクトシダーゼは血液幹細胞移植後の成体型赤芽球造血を同定する有用なマーカーである

Sadaki Asari[1,3], Seiji Okada[2], Yusuke Ohkubo[1], Akemi Sakamoto[1], Masafumi Arima[1], Masahiko Hatano[1], Yoshikazu Kuroda[3], and Takeshi Tokuhisa[1,4]

[1]Department of Developmental Genetics (H2), Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan.

[2]Division of Hematopoiesis, Center for AIDS Research, Kumamoto University, Kumamoto, 860-0811, Japan.

[3]Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kobe University, Kobe 650-0017, Japan.

Key Words: Hematopoiesis, β-galactosidase, stem cells, erythropoiesis.

Word Count: Abstract, 250 Words; Text, 2128 Words.

[4]Address correspondence to: Takeshi Tokuhisa, M.D., Department of Developmental Genetics (H2), Graduate School of Medicine, Chiba University, Inohana 1-8-1, Chuo-ku, Chiba 260-8670, Japan.

Tel: 81-43-226-2181, Fax: 81-43-226-2183, E-mail: tokuhisa@med.m.chiba-u.ac.jp

Footnotes

This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, Technology, Sports and Culture of Japan.

Abbreviations

Ab, antibody; APC, allophycocyanin; β-gal, β-galactosidase; BM, bone marrow; GFP, green fluorescence protein; Lin, lineage markers; PE, phycoerythrin.

ABSTRACT

Background. **Hematopoietic reconstitution after stem cell transplantation has been analyzed using stem cells of Ly5 congenic mice. However, the early erythropoiesis has never been analyzed since this marker is not expressed on all of erythroid lineage cells. The transgenic mouse expressing β-galactosidase (β-gal) or green fluorescence protein (GFP) has been reported. Using these markers, we analyzed the early erythropoiesis after stem cell transplantation.**

Methods. **The β-gal activity and GFP were examined in hematopoietic cells of ROSA26 and GFP transgenic mice, respectively, by a flow cytometry. The primitive hematopoeitic stem cell fraction ($Lin^-c\text{-}kit^+Sca\text{-}1^+$) in bone marrow cells of ROSA26 mice was transferred into lethally irradiated mice. Kinetics of hematopoietic reconstitution was analyzed in the bone marrow and spleen after transplantation.**

Results. **The β-gal activity but not the GFP and Ly5 was detected in all of erythroid ($TER119^+$) cells. The β-gal activity was also detected in the donor derived myeloid ($Mac\text{-}1^+$), B lymphoid ($B220^+$) and T lymphoid ($Thy\text{-}1^+$) cells in the bone marrow and spleen after stem cell transplantation. Kinetics of the hematopoietic reconstitution demonstrated that early erythroid ($TER119^{low}CD71^{med}$) cells were developed in the bone marrow and spleen within 2 days after transplantation before development of proerythroblasts ($TER119^+CD71^{high}$), and that massive erythropoiesis and myelopoiesis were observed in the spleen until 2 and 4 weeks after transplantation, respectively.**

Conclusions. **The β-gal of ROSA26 mice can be a useful marker to identify the donor derived hematopoietic cells including early erythroid cells and the first major wave of erythropoiesis occurs in the spleen after stem cell transplantation.**

INTRODUCTION

The first wave of erythropoiesis in embryos originates in the yolk sac (*1-6*). This is replaced by definitive erythropoiesis in the fetal liver during mouse embryogenesis (*7,8*). The definitive erythropoiesis is also carried out in the bone marrow (BM) and spleen of adult mice (*8*). Several erythroid precursor differentiation stages in the definitive erythropoiesis have been recognized and defined morphologically, based on a gradual decrease in cell volume, increasing chromatin condensation and hemoglobinization as terminal differentiation proceeds (*9*). These are proerythroblasts, basophilic and polychromatophilic erythroblasts, and orthochromatophilic erythroblasts, which give rise to reticulocytes by enucleation. The cell-surface erythroid-specific TER119 antigen is expressed on these erythroblasts (*10*). Conversely, the transferrin receptor (CD71), which is not erythroid-specific, is expressed at very high levels by early erythroid precursors, principally proerythroblasts and early basophilic erythroblasts (*11*), and its levels decrease with erythroid maturation. Therefore, simultaneous immunostaining for TER119 and CD71 can identify these differentiating erythroblasts by flow cytometric analysis.

This definitive erythropoiesis in adult mice can be reproduced by transplantation of hematopoietic stem cells into lethally irradiated syngeneic mice (*12-15*). After hematopoietic stem cell transplantation, the donor-specific erythropoiesis has to be distinguished from erythropoiesis derived from the host stem cells. The Ly5 surface allotype marker system (*16,17*) has been generally used for the

identification of donor-specific hematopoietic cells. Although Ly5 can be detected on almost all of hematopoietic lineage cells (*18*), erythroid cells especially TER119^{+} erythroblasts do not express Ly5 on their cell surface (*10*). Thus, a new cell marker for early erythroid cells has to be discovered for chasing the definitive erythropoiesis after heamtopoeitic stem cell transplantation. Since the ROSA26 (*19*) and green fluorescence protein (GFP) (*20*) transgenic mouse expressing β-galactosidase (β-gal) and GFP, respectively, has been reported, we examined whether these markers could be detected in all of erythroid lineage cells. Here we show that the β-gal activity but not the GFP was detected in all of hematopoietic cells including erythroid (TER119^{+}) cells. Using hematopoietic stem cells of ROSA26 mice, early erythroid (TER119^{low}CD71^{med}) cells were developed in the BM and spleen within 2 days after transplantation before development of proerythroblasts (TER119^{+}CD71^{high}). We discuss the usefulness of ROSA26 mice for investigating the definitive erythropoiesis after hematopoietic stem cell transplantation.

MATERIALS AND METHODS

Mice

C57BL/6 (B6-Ly5.2) and C57BL/6 (B6-Ly5.1) mice were purchased from Japan SLC Co. LTD (Hamamatsu, Japan) and Charles River Japan, Inc., respectively. ROSA26 mice (*19*) were purchased from Jackson laboratory (Bar Harbor, ME, USA) GFP transgenic mice (*20*) were kindly provided by Dr. Okabe (Osaka University). Mice were maintained under specific pathogen free conditions in the animal center of Graduate School of Medicine, Chiba University, and used at 8-16-week-old.

β-gal Staining and Flow Cytometry Analysis

The β-gal activity in cells of ROSA26 mice was detected by staining method described previously (*19*). Briefly, cells of ROSA26 mice and working solution containing 2 mM fluorescent di β-D-galactopyranoside (F-1179) (Molecular Probes, Eugene, OR,USA), prewarmed at 37°C for 10 min, were mixed and incubated again at 37°C for one min. Then, the cold staining medium (phosphate-buffered saline, 4% (v/v) fetal calf serum, 10 mM HEPES, pH 7.2) containing 2 μg/ml propidium iodide was added. The β-gal$^+$ cells were analyzed on a FACSCalibur (Becton Dickinson, San Jose, CA).

Isolation of Hematopoietic Stem Cells

A primitive hematopoietic stem cell fraction in BM cells was isolated by the previously described method (*21*). In brief, biotinylated monoclonal antibodies

(Abs) to B220 (RA3-6B2), Mac-1 (M1/70), Gr-1 (RB6-8C5), CD4 (GK1.5), CD8 (53-6.72), and TER119 were purchased from PharMingen (San Diego, CA) and used to detect lineage markers (Lin). Fluorescent isothiocyanate-conjugated anti-Sca-1 (Ly6A/E) Ab and phycoerythrin (PE)-conjugated anti-c-kit Ab were purchased from PharMingen. Biotinylated Abs were visualized using Streptavidin-Cychrome (PharMingen). BM cells of ROSA26 mice were stained with these Abs, and the Lin^- $c\text{-}kit^+Sca\text{-}1^+$ cells were sorted by a FACSVantage (Becton Dickinson, San Jose, CA) and used as a primitive hematopoeitic stem cell fraction.

Competitive or Stem Cell Repopulation Assay

Wild type (B6-Ly5.1) mice were lethally irradiated (8.5 Gy) 24 hours prior to intravenous injection of cell mixture of BM cells of wild type (B6-Ly5.2) ($2x10^6$) and ROSA26 (B6-Ly5.2) ($2x10^6$) mice for the competitive repopulation assay (*22-24*). Wild type (B6-Ly5.2) mice were lethally irradiated (8.5 Gy) 24 hours prior to intravenous injection of $Lin^-c\text{-}kit^+Sca\text{-}1^+$ cells ($2x10^3$) of ROSA26 (B6-Ly5.2) mice for the stem cell repopulation assay. BM and spleen cells of the radiation chimeric mice were analyzed 7 weeks after transplantation for the competitive repopulation assay and 4-84 days after transplantation for the stem cell repopulation assay. These cells were stained with Abs to CD71-PE and biotinylated TER119 followed by avidin- allophycocyanin (APC; erythroid cells), Mac-1-PE and Gr-1-APC (myeloid cells), CD43-PE and B220-APC (B lymphocytes), or CD8-PE and CD4-APC (T lymphocytes) after β-gal staining as described (*25*). The donor cell type fraction ($\beta\text{-}gal^+$) was analyzed on a FACSCalibur.

RESULTS

Expression of the β-gal of ROSA26 Mice in All Hematopoietic Cells including Erythroblasts

In order to find a marker protein that is expressed in erythroid cells, BM cells from ROSA26 mice were stained with Abs to Ly5.2 and each lineage marker (TER119, Mac-1, B220, Thy-1.2) after β-gal staining, and analyzed on a flow cytometry. The β-gal activity was detected in all hematopoietic lineage cells including TER119$^+$ erythroblasts (Fig. 1A). Although allotype of Ly5 was detected on Mac-1$^+$ BM cells and splenic B and T lymphocytes, most of TER119$^+$ erythroblasts in BM cells do not express Ly5 as previously reported (10). The GFP of GFP transgenic mice is also used as a marker for detecting hematopoietic lineage cells (*25,26*). Thus, BM cells from GFP transgenic mice were stained with Abs to each lineage marker and analyzed on a flow cytometry. The GFP was detected in Mac-1$^+$ and B220$^+$ BM cells but not in almost all of TER119$^+$ BM cells (Fig. 1B). These results suggest that the β-gal of ROSA26 mice is a key molecule to identify all hematopoietic lineage cells including TER119$^+$ erythroblasts.

We next examined stem cell activity of BM cells of ROSA26 mice by competitive repopulation assay with BM cells of wild type mice. We reconstituted lethally irradiated wild type (B6-Ly5.1) mice with a 1:1 mixture of BM cells of ROSA26 (B6-Ly5.2) and wild type (B6-Ly5.2) mice. BM and spleen cells of the BM chimeric mice were stained with Abs to Ly5.2 and hematopoietic lineage markers

after β-gal staining. TER119$^+$ cells were detected in 22% and 8.2% of β-gal$^+$ and β-gal$^-$ BM cells, respectively (Fig. 2). However, Ly5.2 was not detected on TER119$^+$ erythroblasts in the BM and spleen cells. We also acquired the similar results about Mac-1$^+$ or B220$^+$ cells in the BM cells, and Mac-1$^+$, B220$^+$, CD4$^+$ or CD8$^+$ cells in the spleen cells. These results suggest that the progenitors, including hematopoietic stem cells, derived from ROSA26 mice were engrafted in hematopoietic organs and undergo commitment to all hematopoietic lineage cells in the presence of competing wild type progenitors. Furthermore, the definitive erythropoiesis of ROSA26 mice may be chased in BM chimeric mice by the β-gal activity.

Hematopoiesis in the BM and Spleen after Transplantation of Stem Cells of ROSA26 Mice

To examine early erythropoiesis in BM and spleen, we transferred the primitive hematopoietic stem cell fraction in BM cells of ROSA26 mice into irradiated C57BL/6 mice. BM and spleen cells after stem cell transplantation were stained with Abs to lineage markers of hematopoietic cells after β-gal staining, and analyzed on a flow cytometry. The percentages of donor-derived (β-gal$^+$) erythroid (TER119$^+$) and myeloid (Mac-1$^+$) cells were 22% and 77% in BM cells 7 days after transplantation, respectively (Fig. 3). Whereas donor-derived B lymphocytes (B220$^+$) were observed in BM cells 10 days after transplantation, donor-derived T lymphocytes (CD4$^+$ or CD8$^+$) were not detected in BM cells by 4 weeks after transplantation (data not shown).

The donor-derived erythroid and myeloid cells were also detected in spleen cells 7 days after transplantation, and expanded in the spleen until 2 and 4 weeks after transplantation, respectively. Although the donor-derived B lymphocytes were observed in BM cells 10 days after transplantation, these cells were expanded in the spleen 2 weeks after transplantation. The donor-derived T lymphocytes were not detected in spleen cells by 4 weeks after transplantation (data not shown) as previously described (*18*). These results indicate that erythroid and myeloid cells simultaneously develop in the BM and spleen, and that B lymphopoiesis occurs later than erythropoiesis and myelopoiesis in the BM after stem cell transplantation.

Growth curves of the donor-derived hematopoiesis in the BM and spleen after stem cell transplantation were shown in Fig. 4. The number of donor-derived erythroid cells increased progressively in the BM and spleen and reached to the peak 2 weeks after transplantation. Swelling of the spleen was recognized at that time (data not shown). Whereas the number of erythroblasts was almost constant in the BM between 10 and 14 days after transplantation, the number in the spleen increased 10-fold during these 4 days. Moreover, many colonies (spleen colony forming units) were detected in the spleen 10 days after transplantation (data not shown) as previously described (*14,27*). The number of erythroblasts in the spleen decreased rapidly 3 weeks after transplantation and stayed the constant level until 3 months after transplantation. However, the number of erythroblasts in the BM did not decrease 3 weeks after transplantation and stayed the constant level until 3 months after transplantation. The similar kinetics was observed in myelopoiesis in the BM

and spleen after stem cell transplantation, and the myelopoiesis in the spleen continued until 4 weeks after transplantation. The numbers in the BM and spleen were reversed 3 months after transplantation. These results suggest that a major organ for acute erythropoiesis and myelopoiesis is the spleen until 2 weeks after transplantation, and then the main organ for hematopoiesis moves from the spleen to the BM.

The growth curves of B lymphocytes in the BM and spleen were different from those of erythroid and myeloid cells after stem cell transplantation. Since B lymphopoiesis occurs in the BM and mature B cells migrate from the BM into the spleen (*28-30*), the numbers of donor-derived B lymphocytes increased similarly in the BM and spleen during 2 weeks after transplantation, and maintained the numbers at the constant level until 3 months after transplantation.

Development of Early Erythroid (TER119^{low}CD71^{med}) Cells in the BM and Spleen after Stem Cell Transplantation

In order to examine early erythropoiesis in irradiated mice transferred with the primitive hematopoietic stem cell fraction in BM cells of ROSA26 mice, BM and spleen cells were stained with Abs to TER119 and CD71 after β-gal staining, and analyzed on a flow cytometry. Donor-derived (β-gal$^+$) TER119$^+$ erythroblasts were detectable in the BM and spleen 2 days after transplantation, and all of the TER119$^+$ cells belonged to the TER119^{low}CD71^{med} fraction (Fig. 5). Since the donor-derived proerythroblasts (TER119$^+$CD71^{high}) developed in the BM and spleen 7 days after

transplantation, the erythroid cells in the TER119^{low}CD71^{med} fraction developed within 2 days after transplantation may be erythroid progenitors

The donor-derived (β-gal^{+}) TER119^{+}CD71^{high} proerythroblasts enormously expanded in the spleen but a little in the BM 10 days after transplantation and the expansion in the spleen continued until 14 days after transplantation. These results also support that the spleen is a major organ for erythropoiesis in the early recovery phase after stem cell transplantation. The percentages of proerythroblasts increased in the BM and spleen until 2 weeks after transplantation, and then erythroid cells in the TER119^{+}CD71$^{med\sim low}$ fraction increased in the BM and spleen thereafter. Since erythroid cells in the TER119^{+}CD71^{high} fraction proliferate well (*31-33*), these results are reflected to the results shown in Fig. 4 in that the number of erythroid cells increased dramatically in the BM and spleen between 7 and 14 days after transplantation.

DISCCUSSION

A donor cell specific marker is essential for studies on hematopoiesis in chimeric mice after hematopoietic stem cell transplantation. Although allotype of Ly5 is known to be a marker for detecting hematopoietic lineage cells (*16-18*), all of $TER119^+$ erythroblasts in BM cells do not express Ly5 (Fig. 1A). Ubiquitous GFP expression was also reported in GFP transgenic mice (*20*). However, majority of $TER119^+$ erythroblasts in the BM cells did not express GFP (Fig. 1B). Here we demonstrated that β-gal of ROSA26 mice can be a marker to identify all of hematopoietic cells including $TER119^+$ erythroblasts in the BM cells. The ROSA26 mouse strain was produced by random retroviral gene trapping in mouse embryonic stem cells (*34*). An 800-bp promoter fragment isolated from the *β-gal* integration site in ROSA26 mice directs ubiquitous expession of the *EGFP* gene during embryonic and postnatal development in mouse and rat (*35*). Since GFP transgenic mice carry the *EGFP* cDNA under the control of a chicken *β-actin* promoter and cytomegalovirus enhancer (*20*), difference of the exogenous gene expression in $TER119^+$ erythroblasts between ROSA26 and GFP transgenic mice may be due to activity of the promoter of each transgenic mouse. In addition, ROSA26 mice may take advantage of analyzing other cell types such as dendritic cells whose origins do not lend themselves to analysis on the basis of a Ly5 allotype (*19*). Therefore, β-gal of ROSA26 mice is a useful marker to identify donor derived hematopietic cells after stem cell transplantation.

We analyzed early hematopoiesis including erythropoiesis in the BM and spleen after transplantation of hematopoietic stem cells of ROSA26 mice. This system allowed quantitative analyses of hematopoiesis in the BM and spleen by a flow cytometry. Then, we demonstrated that a major organ for erythropoiesis and myelopoiesis in the acute recovary phase was the spleen until 2 weeks after transplantation, and that the organ moved from the spleen to the BM thereafter. These results are also supported by the previous results that progenitors of hematopoietic lineage cells accumulate rapidly in the spleen after BM cell transfer (*36,37*).

Using the transplantation system with ROSA26 stem cells, we further discovered early erythroid progenitors in the $TER119^{low}CD71^{med}$ fraction, since the $TER119^{low}CD71^{med}$ fraction was detected in the BM and spleen within 2 days after transplantation and the donor-derived proerythroblasts ($TER119^{+}CD71^{high}$) developed in the BM and spleen 7 days after transplantation. TER119 is expressed on all of erythroblasts including proerythroblasts (*10*), and CD71 is expressed at very high levels on early erythroid precursors (*11*). Further studies demonstrated that double staining of BM and spleen cells with Abs to TER119 and CD71 can distinguish proerythroblasts ($TER119^{+}CD71^{high}$) from the other erythroblasts ($TER119^{+}CD71^{med\text{-}low}$) by the amount of CD71 expression (*31*). Zhang *et al.* divided E14.5 fetal liver cells into 5 distinct populations ($TER119^{low}CD71^{med}$, $TER119^{low}CD71^{high}$, $TER119^{high}CD71^{high}$, $TER119^{high}CD71^{med}$ and $TER119^{high}CD71^{low}$) using Abs to TER119 and CD71 (*38*). They indicated that approximately 40% of

sorted TER119^{low}CD71^{med} cells were colony forming unit erythroblasts (CFU-E) progenitors and that the percentage of CFU-E progeniters among TER119^{low}CD71^{med} cells was about 5-fold higher than that of total fetal liver cells. Thus, these TER119^{low}CD71^{med} cells may be equivalent to burst forming unit erythroblasts (BFU-E) and/or CFU-E in the *in vitro* colony forming assay (*39*). Taken together, β-gal of ROSA26 mice is a useful marker for donor derived hematopoietic cells including erythroid lineage cells in chimeric mice after stem cell transplantation.

CONCLUSION

The definitive erythropoiesis can be chased in irradiated mice transferred with the primitive hematopoietic stem cell fraction in BM cells of ROSA26 mice by FACS analysis, and the erythroid progenitors identified in the TER119^{low}CD71^{med} fraction are developed in the BM and spleen within 2 days after transplantation before development of proerythroblasts (TER119^{+}CD71^{high}). Furthermore, the majority of erythropoiesis and myelopoiesis in the acute recovery phase occurs in the spleen until 2 weeks after stem cell transplantation.

Acknowledgments

We thank H. Satake for skillful technical assistance and N. Kakinuma for secretarial services.

REFERENCES

1. Dzierzak E, Medvinsky A. Mouse embryonic hematopoiesis. Trends Genet 1995; 11: 359.

2. Medvinsky A, Dzierzak E. Development of the definitive hematopoietic hierarchy in the mouse. Dev Comp Immunol 1998; 22: 289.

3. Medvinsky A, Dzierzak E. Definitive hematopoiesis is autonomously initiated by the AGM region. Cell 1996; 86: 897.

4. Palis J, Robertson S, Kennedy M, Wall C, Keller G. Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. Development 1999; 126: 5073.

5. Orkin SH. Development of the hematopoietic system. Curr Opin Genet Dev 1996; 6: 597.

6. Palis J, Yoder MC. Yolk-sac hematopoiesis: the first blood cells of mouse and man. Exp Hematol 2001; 29: 927.

7. Palis J, Segel GB. Developmental biology of erythropoiesis. Blood Rev 1998; 12: 106.

8. Dame C, Juul SE. The switch from fetal to adult erythropoiesis. Clin Perinatol 2000; 27: 507.

9. Fawcett DW, Jensh RP. Hemopoiesis. In: Bloom & Fawcett, ed. Concise histology. New York, Chapman and Hall 1997, pp 84.

10. Kina T, Ikuta K, Takayama E, et al. The monoclonal antibody TER-119 recognizes a molecule associated with glycophorin A and specifically marks

the late stages of murine erythroid lineage. Br J Haematol 2000; 109: 280.

11. Ponka P, Nam-lok C. Identification of an erythroid active element in the transferrin receptor gene. J Biol Chem 2000; 275: 24185.

12. Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. Science 1988; 241: 58.

13. Okada S, Nakauchi H, Nagayoshi K, Nishikawa S, Miura Y, Suda T. In vivo and in vitro stem cell function of c-kit- and Sca-1-positive murine hematopoietic cells. Blood 1992; 15: 3044.

14. Ploemacher RE, Brons RH. Separation of CFU-S from primitive cells responsible for reconstitution of the bone marrow hemopoietic stem cell compartment following irradiation: evidence for a pre-CFU-S cell. Exp Hematol 1989; 17: 263.

15. Okada S, Nakauchi H, Nagayoshi K, et al. Enrichment and characterization of murine hematopoietic stem cells that express c-kit molecule. Blood 1991; 78: 1706.

16. Shen FW, Tung JS, Boyse EA. Further definition of the Ly-5 system. Immunogenetics 1986; 24: 146.

17. Morse HC 3rd, Shen FW, Hammerling U. Genetic nomenclature for loci controlling mouse lymphocyte antigens. Immunogenetics 1987; 25: 71.

18. Okada S, Nagayoshi K, Nakauchi H, Nishikawa S, Miura Y, Suda T. Sequential analysis of hematopoietic reconstitution achieved by transplantation of hematopoietic stem cells. Blood 1993; 81: 1720.

19. Zambrowicz BP, Imamoto A, Fiering S, Herzenberg LA, Kerr WG, Soriano P. Disruption of overlapping transcripts in the ROSA beta geo 26 gene trap strain

leads to widespread expression of beta-galactosidase in mouse embryos and hematopoietic cells. Proc Natl Acad Sci USA 1997; 94: 3789.

20. Okabe M, Ikawa M, Kominami K, et al. 'Green mice' as a source of ubiquitous green cells. FEBS Lett. 1997; 407: 313.

21. Okada S, Fukuda T, Inada K, Tokuhisa T. Prolonged expression of c-fos suppresses cell cycle entry of dormant hematopoietic stem cells. Blood 1999; 93: 816.

22. Szilvassy SJ, Humphries RK, Lansdorp PM, Eaves AC, Eaves CJ. Quantitative assay for totipotent reconstituting hematopoietic stem cells by a competitive repopulation strategy. Proc Natl Acad Sci USA 1990; 87: 8736.

23. Souabni A, Cobaleda C, Schebesta M, Busslinger M. Pax5 promotes B lymphopoiesis and blocks T cell development by repressing Notch1. Immunity 2002; 17: 781.

24. Bunting KD, Bradley HL, Hawley TS, Moriggl R, Sorrentino BP, Ihle JN. Reduced lymphomyeloid repopulating activity from adult bone marrow and fetal liver of mice lacking expression of STAT5. Blood 2002; 99: 479.

25. Migishima F, Oikawa A, Kondo S, et al. Full reconstitution of hematopoietic system by murine umbilical cord blood. Transplantation 2003; 75: 1820.

26. Hayakawa J, Migita M, Ueda T, Shimada T, Fukunaga Y. Generation of a chimeric mouse reconstitution with green fluorescent protein-positive bone marrow cells: A useful model for studying the behavior of bone marrow cells in regeneration in vivo. Int J Hematol 2003; 77: 456.

27. Till JE, McCulloch EA. A direct measurement of radiation sensitivity of normal mouse bone marrow cells. Radiat Res 1961; 14: 213.

28. Imhof BA, Dunon D. Leukocyte migration and adhesion. Adv Immunol 1995; 58: 345.

29. Springer TA. Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. Annu Rev Physiol 1995; 57: 827.

30. Yednock TA, Rosen SD. Lymphocyte homing. Adv Immunol 1989; 44: 313.

31. Socolovsky M, Nam H, Fleming MD, Haase VH, Brugnara C, Lodish HF. Ineffective erythropoiesis in Stat5a(-/-)5b(-/-) mice due to decreased survival of early erythroblasts. Blood 2001; 98: 3261.

32. Ponka P. Tissue-Specific Regulation of Iron Metabolism and Heme Synthesis: Distinct Control Mechanisms in Erythroid Cells. Blood 1997; 89: 1.

33. Zhang D, Johnson MM, Miller CP, Pircher TJ, Geiger JN, Wojchowski DM. An optimized system for studies of EPO-dependent murine pro-erythroblast development. Exp Hematol 2001; 29: 1278.

34. Friedrich G, Soriano P. Promoter traps in embryonic stem cells: a genetic screen to identify and mutate developmental genes in mice. Genes Dev 1991; 5: 1513.

35. Kisseberth WC, Brettingen NT, Lohse JK, Sandgren EP. Ubiquitous expression of marker transgenes in mice and rats. Dev Biol 1999; 214: 128.

36. Szilvassy SJ, Meyerrose TE, Ragland PL, Grimes B. Differential homing and engraftment properties of hematopoietic progenitor cells from murine bone marrow, mobilized peripheral blood, and fetal liver. Blood 2001; 98: 2108.

37. Szilvassy SJ, Bass MJ, Van Zant G, Grimes B. Organ-selective homing defines engraftment kinetics of murine hematopoietic stem cells and is compromised by Ex vivo expansion. Blood 1999; 93: 1557.

38. Zhang J, Socolovsky M, Gross AW, Lodish HF. Role of Ras signaling in erythroid differentiation of mouse fetal liver cells: functional analysis by a flow cytometry-based novel culture system. Blood 2003; 102: 3938.

39. Heath DS, Axelrad AA, McLeod DL, Shreeve MM. Separation of the erythropoietin-responsive progenitors BFU-E and CFU-E in mouse bone marrow by unit gravity sedimentation. Blood 1976; 47: 777.

FIGURE LEGENDS

Figure 1. Expression of the β-gal of ROSA26 mice but not the GFP of GFP transgenic mice in all hematopoietic cells including TER119$^+$ erythroblasts. (A) BM cells from ROSA26 and wild type mice were stained with Abs to Ly5.2 and each lineage marker (TER119, Mac-1, B220, Thy-1.2) after β-gal staining. (B) BM cells from GFP transgenic mice were stained with Abs to each lineage marker (TER119, Mac-1 or B220). These cells were analyzed on a flow cytometry. These results are representative of two (both A and B) independent experiments.

Figure 2. Analysis of stem cell activity of BM cells of ROSA26 mice by competitive repopulation assay. BM cells of ROSA26 (B6-Ly5.2) and wild type (B6-Ly5.2) mice were used at a ratio of 1:1 to reconstitute lethally irradiated wild type (B6-Ly5.1) mice. BM and spleen cells of a representative BM chimeric mouse were stained with Abs to Ly5.2 and each lineage marker (TER119, Mac-1, B220, CD4 or CD8) after β-gal staining. These cells were analyzed on a flow cytometry 7 weeks after transplantation. The numbers in the corners indicate the percentages of cells in each quadrant. These results are representative of three independent experiments.

Figure 3. Kinetic analysis of hematopoietic reconstitution in the BM and spleen after stem cell transplantation. The primitive hematopoietic stem cell fraction (Lin$^-$c-kit$^+$Sca-1$^+$ cells ($2x10^3$)) in BM cells of ROSA26 mice was transferred intravenously into lethally irradiated C57BL/6 mice. At various times after transplantation, BM and spleen cells of a representative mouse were stained with Abs to each lineage marker

(TER119, Mac-1 or B220) after β-gal staining. These cells were analyzed on a flow cytometry. The numbers in the corners indicate the percentages of cells in each quadrant. These results are representative of three independent experiments.

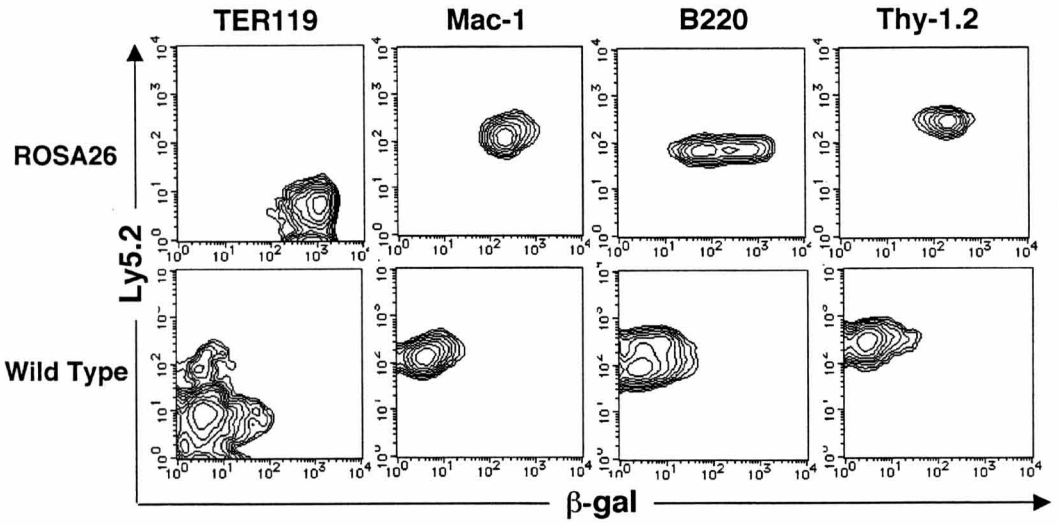
Figure 4. Recovery of hematopoietic cells in the BM and spleen after stem cell transplantation. The primitive hematopoietic stem cell fraction ($Lin^-c\text{-}kit^+Sca\text{-}1^+$ cells ($2x10^3$)) in BM cells of ROSA26 mice was transferred intravenously into lethally irradiated C57BL/6 mice. At various times after transplantation, BM and spleen cells of a representative mouse were stained with Abs to each lineage marker (TER119, Mac-1 or B220) after β-gal staining. These cells were analyzed on a flow cytometry. The absolute numbers of donor derived each cell lineage in the BM (closed circle) and spleen (open square), calculated by the percentages of each cell lineage and the BM and spleen cell numbers, are shown at various times after stem cell transplantation. (A) Definitive erythropoiesis, (B) myelopoiesis and (C) B lymphopoiesis are shown. These results are representative of three independent experiments. Data are mean ± SD from three mice.

Figure 5. Kinetic analysis of erythropoietic reconstitution in the BM and spleen after stem cell transplantation. The primitive hematopoietic stem cell fraction ($Lin^-c\text{-}kit^+Sca\text{-}1^+$ cells ($2x10^3$)) in BM cells of ROSA26 mice was transferred intravenously into lethally irradiated C57BL/6 mice. At various times after transplantation, BM and spleen cells were stained with Abs to TER119 and CD71 after β-gal staining. These cells were analyzed on a flow cytometry. The numbers indicate the percentages of cells in each bar or square. These results are representative of three independent

experiments.

Figure 1

A)


TER119
Mac-1
B220
Thy-1.2
ROSA26
Wild Type
Ly5.2
β-gal

B)

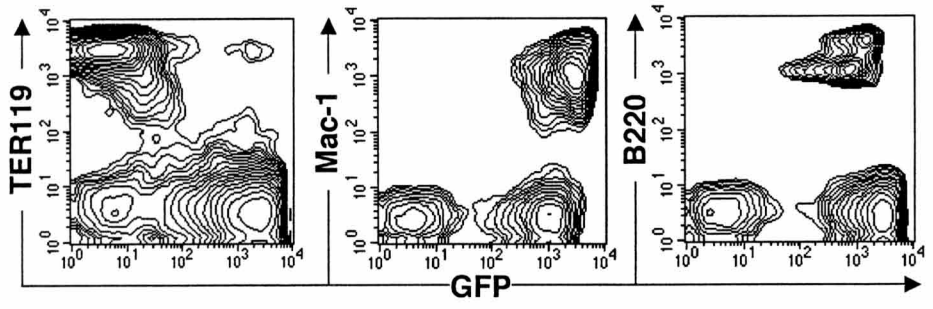

TER119
Mac-1
B220
GFP

Figure 2

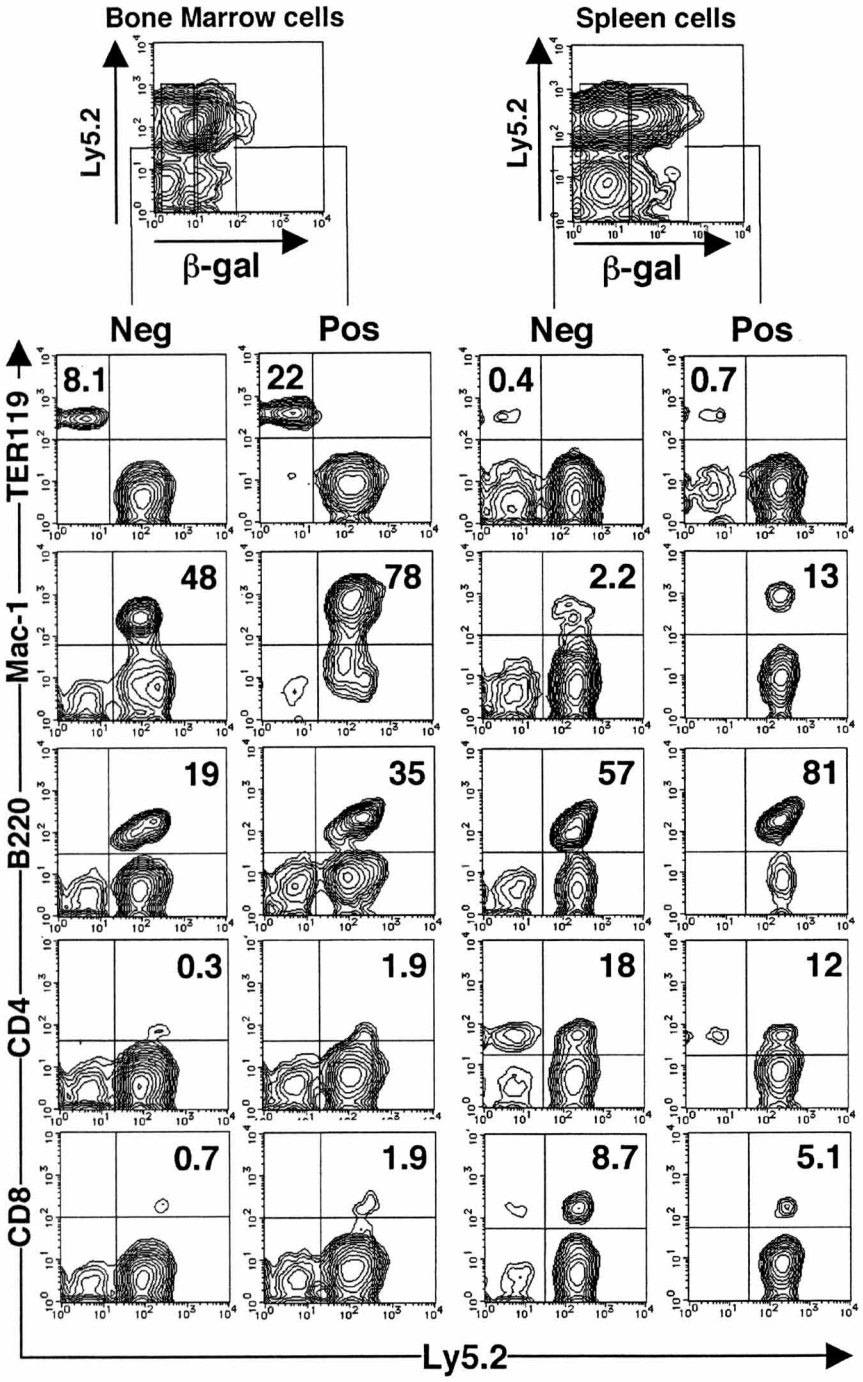

Bone Marrow cells
Spleen cells
Ly5.2
β-gal
Neg
Pos
TER119
Mac-1
B220
CD4
CD8
Ly5.2
8.1
22
0.4
0.7
48
78
2.2
13
19
35
57
81
0.3
1.9
18
12
0.7
1.9
8.7
5.1

Figure 3

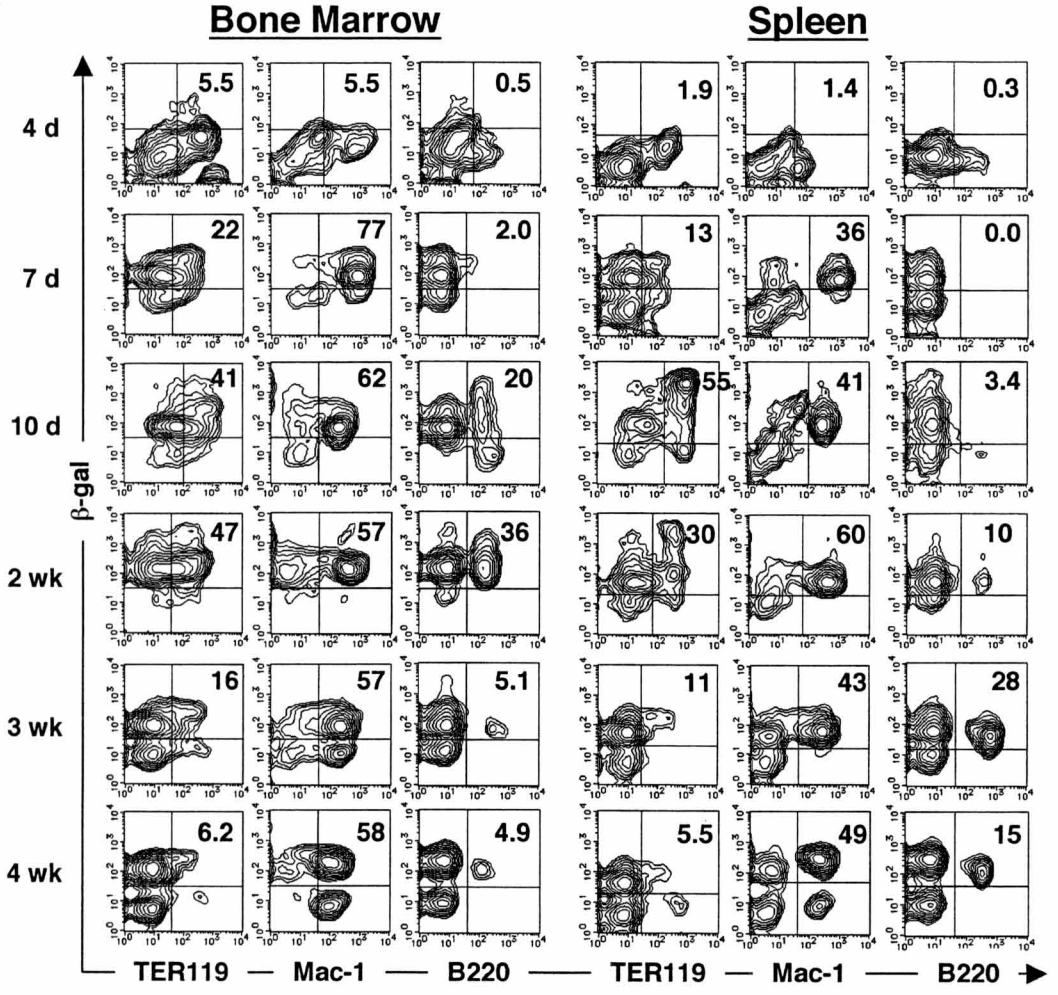

Bone Marrow
Spleen
4 d
7 d
10 d
2 wk
3 wk
4 wk
β-gal
5.5
5.5
0.5
1.9
1.4
0.3
22
77
2.0
13
36
0.0
41
62
20
55
41
3.4
47
57
36
30
60
10
16
57
5.1
11
43
28
6.2
58
4.9
5.5
49
15
TER119
Mac-1
B220
TER119
Mac-1
B220

Figure 4

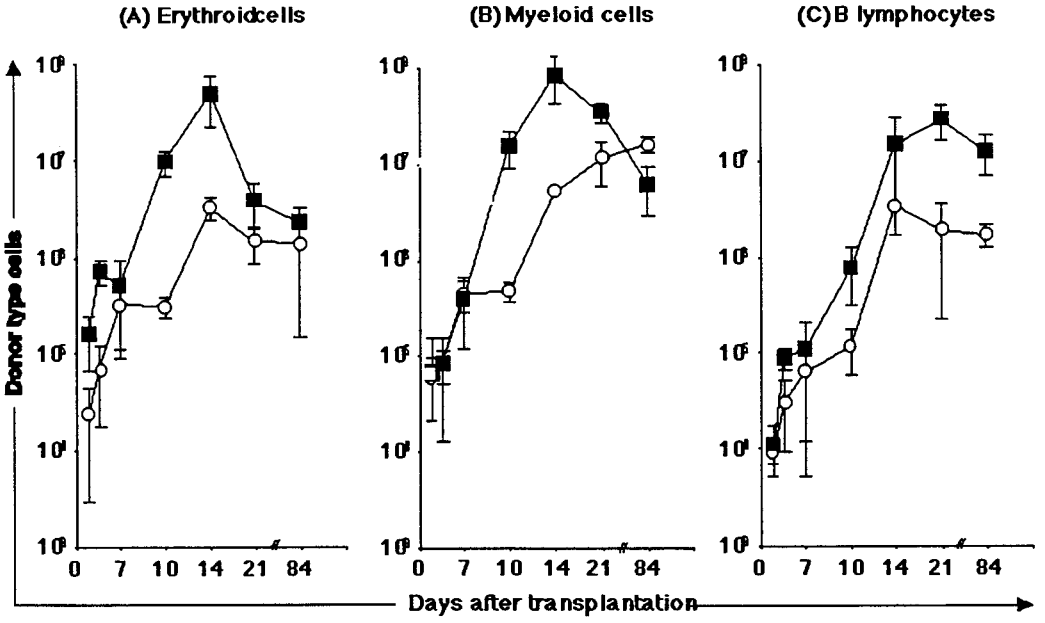
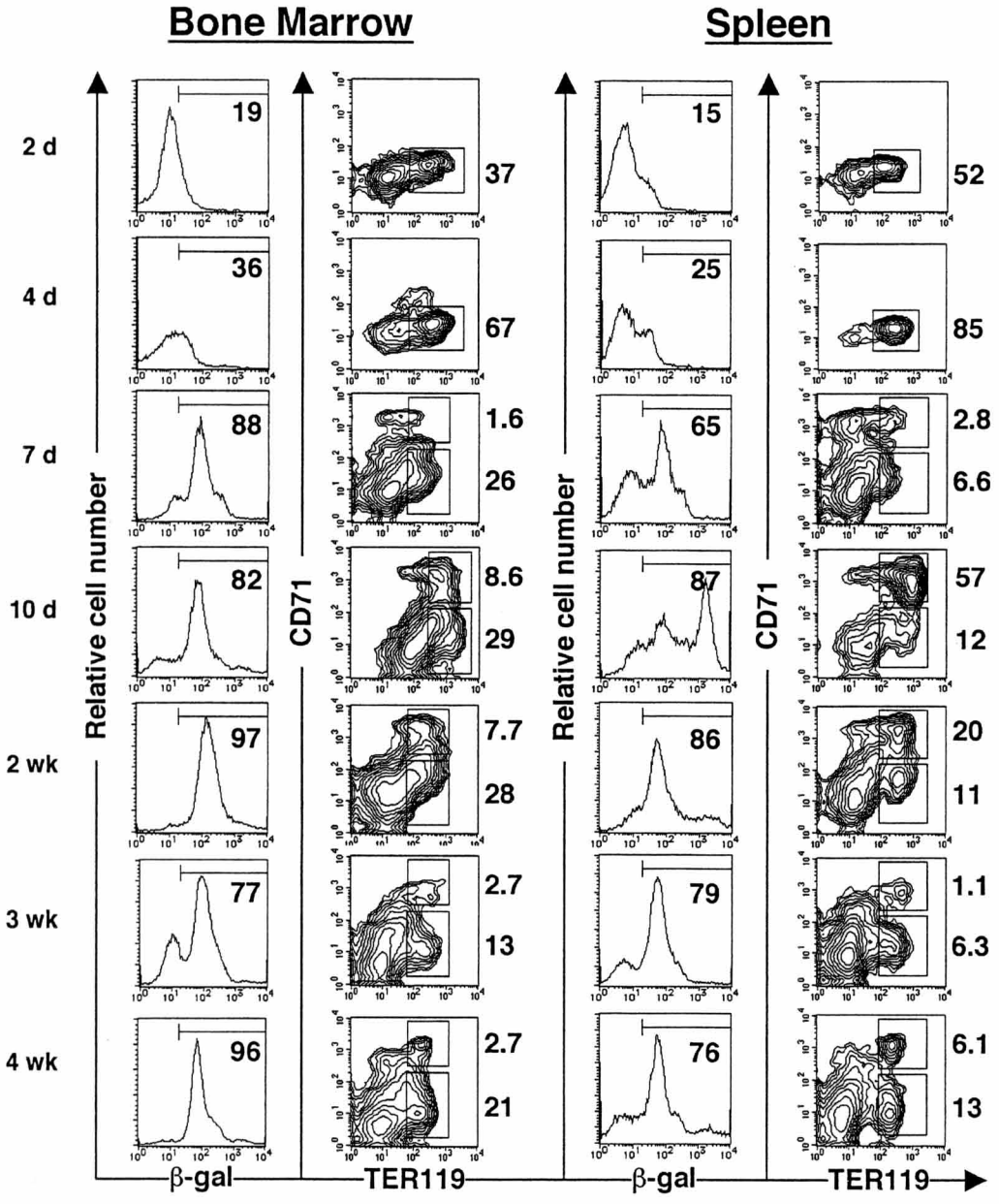

(A) Erythroidcells
(B) Myeloid cells
(C) B lymphocytes
Donor type cells
0 7 10 14 21 84
Days after transplantation

Figure 5


Bone Marrow
Spleen
2 d
4 d
7 d
10 d
2 wk
3 wk
4 wk
Relative cell number
CD71
β-gal
TER119
19
37
36
67
88
1.6
26
82
8.6
29
97
7.7
28
77
2.7
13
96
2.7
21
15
52
25
85
65
2.8
6.6
87
57
12
86
20
11
79
1.1
6.3
76
6.1
13