Serial Transplantation of p53-Deficient Hemopoietic Progenitor Cells to Assess Their Infinite Growth Potential

YOKO HIRABAYASHI,[1]* MOTOI MATSUDA,* SHIN-ICHI AIZAWA,† YUKIO KODAMA,* JUN KANNO,* and TOHRU INOUE*

**Cellular and Molecular Toxicology Division, Center for Biological Safety Research, National Institute of Health Sciences, 1-18-1 Kamioyhga, Setagayaku, Tokyo-158-8501; and †Institute of Genetics and Developmental Biology, Kumamoto University Medical School, 4-24-1 Kyuhinji, Kumamoto 862-0976, Japan*

Thirty-five years ago, Siminovitch et al. (Siminovitch L, Till JE, McCulloch EA. J Cell Com Physiol 64:23–32, 1964), using serially transplanted mouse spleens at 14-day intervals, observed a markedly progressive decline in the proliferative capacity of bone marrow (BM) cells, with the loss of clonogenicity by the fourth transplant generation. Using the same protocol, we assessed the proliferative capacity of p53-deficient mouse BM cells transplanted serially at the same 14-day intervals into lethally irradiated mice, which was a useful tool for understanding the characteristics of hemopoietic stem cells lacking solely the *p53* gene function. BM cells from p53-deficient homozygous ($p53^{-/-}$), p53-heterozygous ($p53^{+/-}$), and wild-type ($p53^{+/+}$) C57BL/6 mice were transplanted into lethally irradiated C57BL/6 recipients. Fourteen days later, the repopulated spleens were harvested, and 10^7 cells were retransplanted into secondary recipients. Serial transplantation was continued at 14-day intervals until hemopoietic repopulation failure. The number of heterozygous and homozygous p53-deficient spleen cells increased logarithmically up to the fourth and fifth passages, respectively, whereas wild-type spleen cells ceased to proliferate by the third passage. The number of macroscopic spleen colonies increased logarithmically until the third passage in recipients of heterozygous and homozygous p53-deficient cells, but ceased to grow by the second passage in recipients of wild-type cells. The numbers of heterozygous and homozygous p53-deficient colony forming units in spleen (CFUs-S) remained stable during the first four transplant generations, whereas that of wild-type CFUs-S decreased progressively from the first transplant generation onward. The clonogenicity of p53-deficient cells was lost when the number of CFUs-S per spleen decreased to below 10. This suggests that one out of 10 CFUs-S might be long-term repopulating cells (LTRCs), and that p53-deficient LTRCs may proliferate more rapidly than wild-type LTRCs. Longer passages that were possible in the p53-deficient groups were considered to be due to the faster cell cycle of the p53-deficient hemopoietic progenitor cells, as determined by bromodeoxyuridine incorporation with purging by UV light exposure, followed by hemopoietic colony assay (BUUV assay).

[Exp Biol Med Vol. 227(7):474–479, 2002]

Key words: p53-deficient hemopoietic stem cells; serial bone marrow transplant; infinite; stem cell aging; BUUV assay

In the early 1960s, several investigators demonstrated that mice could survive lethal irradiation when given a histocompatible bone marrow transplant (BMT) (1–5). Moreover, they found that serial transplantation of donor BM cells was possible, provided that the initial donor inocula contained more than 10^6 cells. However, as shown by Harrison *et al.* (6–8), the proliferative potential of donor BM cells was reduced after 100 months of serial passages at 3-month intervals; thus, with repeated transplantations, the number of BM cells was found to be reduced.

On the other hand, when Siminovitch *et al.* (9) attempted to reduce the interval to 14 days, they found that the number of donor-derived cells declined markedly with each passage, and the cells failed to engraft by the fourth transplant generation. This finding on the serial transplantation into the lethally irradiated recipients' hemopoietic system suggests that the transplantability of BM cells is a function of time with respect to stem cell recovery after BMT.

We raised the questions: If stem cell proliferation were accelerated, would serial transplantation at 14-day intervals be successful, and would these stem cells proliferate sufficiently to allow continuous serial transplantation? To answer these questions, we proliferated hemopoietic progenitor cells *in vitro* as well as *in vivo*, modifying Siminovitch and coworkers' (9) protocol to determine the factors limit-

This work was supported in part by grants from the Ministry of Education Culture, Sports, Sciences and Technology, Japan.

[1] To whom requests for reprints should be addressed at Cellular and Molecular Toxicology Division, National Institute of Health Sciences, 1-18-1 Kamiyohga, Setagayaku, Tokyo-158-8501, Japan. E-mail: yokohira@nihs.go.jp

Received September 18, 2001.
Accepted March 11, 2002.

1535-3702/02/2277-0474$15.00

Copyright © 2002 by the Society for Experimental Biology and Medicine

ing sustained long-term repopulation. The answers could be crucial for determining not only the prognosis of BMT recipients in the clinic, but also the characteristics of the continuously transplantable BM stem cell population.

In an earlier study (Hirabayashi Y, Matsuda M, Aizawa S-I, Kodama Y, Kanno J, Inoue T, unpublished data), we found that p53-deficient BM-derived CFUs-S proliferate more rapidly and have a more accelerated cell cycle than wild-type CFUs-S. Therefore, p53-deficient BM cells were used in the present study to assess the proliferative capacity and transplantability of serially passaged BM-derived stem cells.

Materials and Methods

Mice. All animal experiments were conducted with the approval of the Animal Care and Use Committee of the National Institute of Health Sciences, Japan. p53 knockout (KO) mice ($p53^{-/-}$) were established by gene targeting of embryonic stem cells from $(DBA \times C57BL/6)F_1$ as described by Tsukada *et al.* (10). Heterozygous p53-deficient ($p53^{+/-}$) mice, provided by Drs. Tsukada and Aizawa, were backcrossed with wild-type ($p53^{+/+}$) C57BL/6 mice for seven generations at the animal facility of the National Institute of Health Sciences of Japan. Eight-week-old p53-deficient ($p53^{-/-}$ and $p53^{+/-}$) male mice as well as wild-type ($p53^{+/+}$) male littermates of $p53^{+/-}$ mice were used as BM donors. Eight-week-old C57BL/6 male mice from Japan Shizuoka Laboratory Animal Center (SLC; Hamamatsu, Japan) were used as recipients. All the mice were housed under specific pathogen-free (SPF) conditions using a 12:12-hr light:dark cycle. Autoclaved tap water and food pellets were provided *ad libitum.* The initial passage was started using the pooled BM cells from three mice each of the three genotypes.

Irradiation. Recipient mice were exposed to lethal radiation of 915 cGy delivered at a dose-rate of 124 cGy using a ^{137}Cs-gamma irradiator (Gamma Cell 40; CSR, Toronto, Canada) with a 0.5-mm aluminum-copper filter.

Hemopoietic Repopulation: *First Passage.* BM cells derived from three mice each of the three genotypes were harvested, pooled, suspended in RPMI 1640 (Dainippon Pharmaceutical Co., Osaka, Japan), and processed to obtain a single-cell suspension by repeated aspiration through a 27-gauge hypodermic needle. The cell concentration was adjusted to 5×10^6 cells/ml by dilution with the same medium, and 10^6 BM cells in 0.2-ml aliquots were transplanted into lethally irradiated recipients by tail vein injection (Fig. 1).

Hemopoietic Repopulation: *Second through Sixth Passages.* Cells of a repopulated recipient spleen were harvested 14 days after transplantation. Three spleens from each genotype group were processed to obtain a single-cell suspension and were then pooled. The concentration of spleen cells was adjusted to 5×10^7 cells/ml, and 10^7 cells in 0.2-ml aliquots were transplanted by tail vein injection into lethally irradiated recipients. Because a large number of cells had to be injected at later passages, and in order to inject a consistent number of cells with little variation, special care was required in aspirating cell suspension after a quick pipetting with careful and gentle agitation. Cells were injected immediately after aspirating the cell suspension in order to avoid cell sedimentation.

CFUs-S Assay. The Till and McCulloch method (11) was used to determine the number of CFU-S. Aliquots of BM cell suspensions were also used for evaluating the number of CFU-S in the reference animals. The concentration of cells was adjusted to that appropriate for producing non-confluent spleen colonies, and the cells were then transplanted into lethally irradiated mice by tail vein injection. Spleens were harvested 14 days later and fixed in Bouin's solution. Macroscopic spleen colonies were counted under an inversion microscope at a magnification of ×5.6. For the C57BL/6 strain, irradiated with 915 cGy, all the colonies visible at 14 days are known to have originated from transplanted BM cells (12, 13).

CFU-GM Assay. Granulomacrophage CFU (CFU-GM) were assayed in semisolid methylcellulose culture. Briefly, 8×10^4 BM cells suspended in 100 μl of Dulbecco modified Eagle media (DMEM) were added to 3.9 ml of a culture medium containing 0.8% methylcellulose (Nakarai Tesque Co., Kyoto, Japan), 30% fetal calf serum (HyClone Laboratories Inc., Logan, UT), 1% bovine serum albumin (BSA; Sigma, St. Louis, MO), 10^{-4} M mercaptoethanol (Sigma), and 10 ng/ml murine granulocyte macrophage

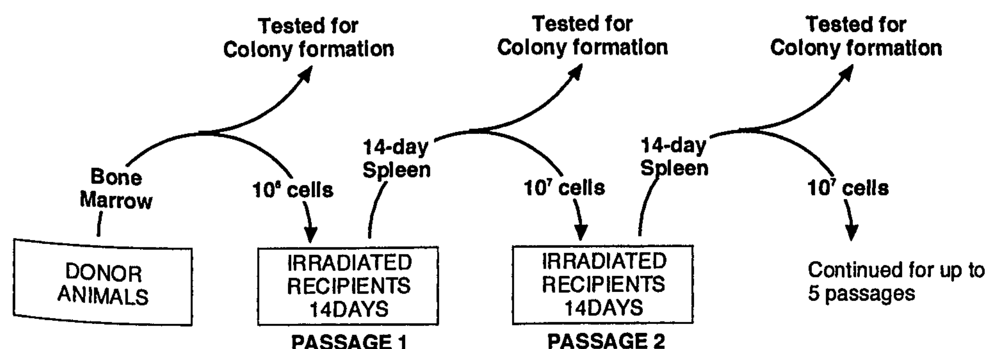

Figure 1. Flow chart of serial transplantation protocol. Original transplants derived from femoral bone marrow cells from three mice per group were transplanted into lethally irradiated recipients through the tail vein. The numbers of cells transplanted for serial passages are shown; however, to avoid the confluency of colonies in the spleen, a much smaller number of cells were injected into reference animals. Both the reference animals and the mice for serial transplantation was sacrificed on Day 14, and colonies were counted. All the procedures followed that of Siminovitch *et al.* (9).

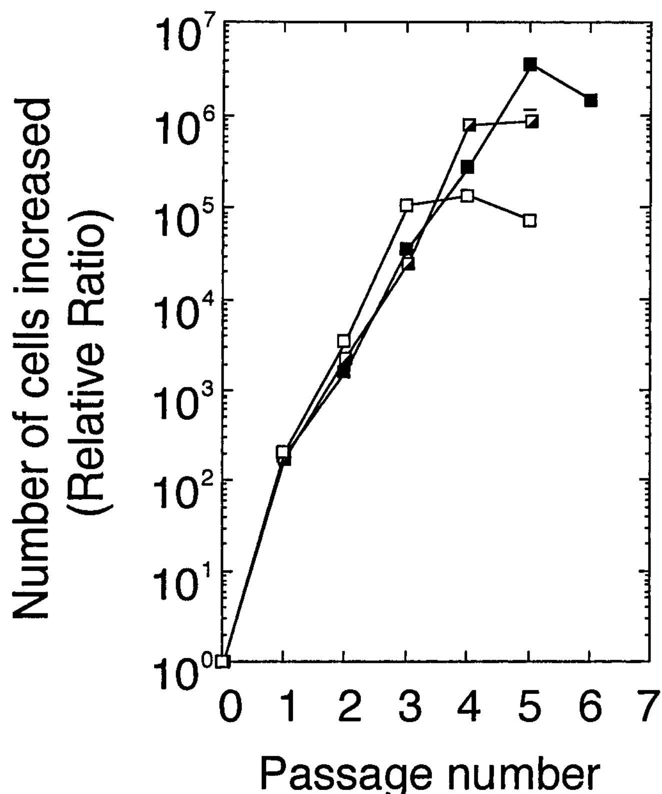

Figure 2. The number of transplanted cells increased during the repopulation assay (relative ratios). The number of cells harvested from spleens at each passage was calculated as a relative ratio to the number of bone marrow cells transfused in the first passage. Fifty percent was the value used as the splenic seeding efficiency (21). Recipient mice were sacrificed and their spleens were removed. Spleen cells were harvested by repeated inflation with a medium and squeezing with forceps, followed by gentle agitation and pipetting in tubes. The entire procedure was performed in an ice-cold medium. For later passages, 10^7 spleen cells were transplanted into lethally irradiated mice, as shown in Figure 1. The number of cells was cumulatively recalculated after each passage, and was plotted on a logarithmic scale. The number of spleen colonies for the reference animals was counted separately, but also 14 days after transplantation, as shown in Figure 3. (-□-, wild type; -◪-, heterozygous deficiency; -■-, homozygous deficiency; vertical bars, standard deviation of mean. The data symbols without error bars indicate that the deviation is within each symbol.).

colony-stimulating factor (GM-CSF; R&D Systems, Minneapolis, MN). One-milliliter aliquots containing 2×10^4 BM or spleen cells were plated in triplicate in a 35-mm tissue-culture plate (Nalgen Nunc International, Rochester, NY), and incubated for 6 days in a completely humidified incubator at 37°C with 5% CO_2 in air. Colonies were counted under an inverted microscope (Olympus Optical Co., Tokyo, Japan).

BUUV assay. The bromodeoxyuridine(BrdUrd)-labeled cells purged by UV light (BUUV) assay for evaluating kinetics of hemopoietic progenitor cells were employed as described elsewhere (14, 15). Briefly, cells in the S phase (DNA synthesis) were labeled *in vivo* with BrdUrd followed by exposure to near-UV light in order to kill cells that incorporated BrdUrd, and then the survival ratio was evaluated based on the number of hemopoietic colonies formed determined by assays such as GM-CFU and CFU-S.

Results

Proliferation of Repopulating Cells. In the repopulation assay shown in Figure 2, the number of transplanted cells initially increased exponentially in all groups when calculated based on the relative increasing ratios. However, proliferation of serially passaged cells derived from the wild-type BM in the recipient spleens was arrested at passage 3, that from the heterozygous p53-deficient BM was arrested at passage 4, and that from the homozygous p53-deficient BM was arrested at passage 5. In contrast, the number of repopulated cells in the recipient BM did not show significant inter-group differences (data not shown). Moreover, the cumulative increase in BM cells transplanted

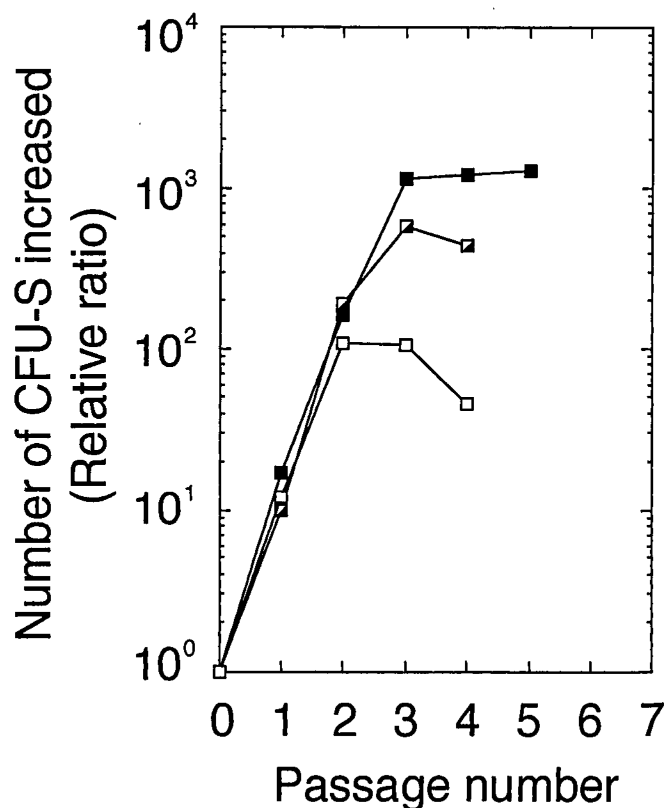

Figure 3. The number of CFU-Ss increased as determined by the repopulation assay (relative ratios). The number of CFU-Ss harvested from spleens at each passage was converted based on the results of CFU-S assays, and was then calculated as a relative ratio to the number of CFU-Ss originally existing in the first BM cells transfused in the first passage. Fifty percent was the value used as the splenic seeding efficiency (21). The number of spleen colonies was determined by a CFU-S assay using aliquots of cell suspensions, and spleen cells were injected into reference animals of the same lot after lethal irradiation. The mice were sacrificed 14 days after BMT. Their spleens were removed, and the colonies were counted after staining with Bouin's fixative. The number of colonies was converted into the actual number of cells used in the serial transplantation, cumulatively recalculated, and then plotted on a logarithmic scale. (-□-, wild type; -◪-, heterozygous deficiency; -■-, homozygous deficiency; vertical bars, standard deviation of mean. The data symbols without error bars indicate that the deviation is within each symbol.)

and the increase in the spleen weight in each passage, calculated based on the growth rate at each passage, were comparable among the three genotypes (data not shown).

Proliferation of CFUs-S. Transplanted CFUs-S manifested an exponential growth (Fig. 3). The calculated CFUs-S derived from wild-type BM repopulated in the recipient spleen, "CFU-S/spleen cell ratio," decreased rapidly, whereas that from the p53-deficient BM remained stable up to the passage 2, after which it decreased (Fig. 4). Furthermore, the rate of decrease in CFUs-S repopulation of hemopoietic progenitor cells in p53-deficient BM was more attenuated in the homozygous than that in the heterozygous p53-deficient BM. Thus, in the homozygous p53-deficient group, the proliferation of transplanted CFU-S was arrested by the fourth passage. Genotype-related differences in the proliferative rates of serially transplanted CFU-S, as reflected by the size and number of spleen colonies, are shown in Figure 5. Thus, the number and size of spleen colonies derived from wild-type BM cells (Fig. 5, top panel) were smaller than those derived from homozygous p53-deficient

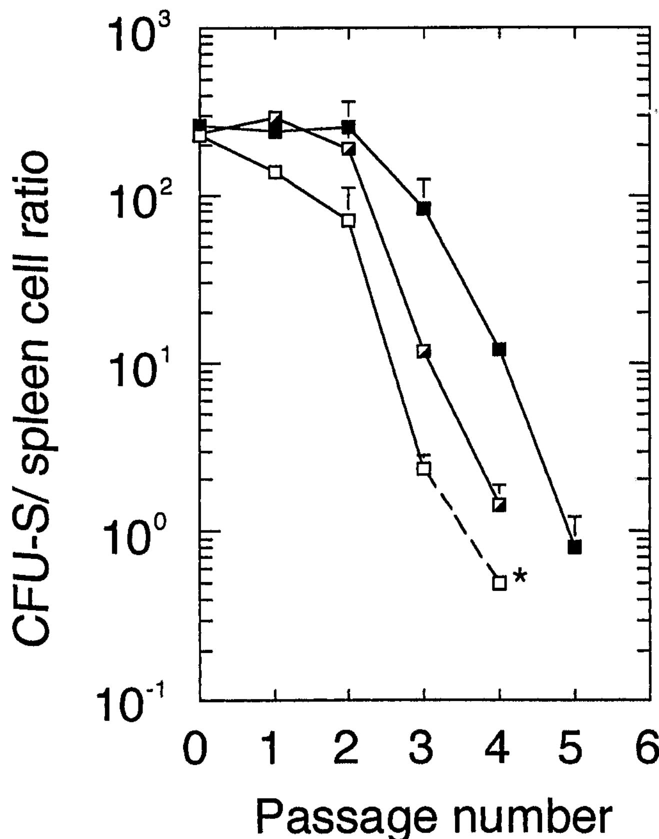

Figure 4. The number of CFU-S in donor-derived spleen cells during passage. The number of spleen cells obtained in Figure 2 and the number of CFUs-S obtained in Figure 3 were used. The number of CFU-S from the wild-type donor in the recipient spleens starts to decrease from the second passage, whereas that from the p53-deficient donor remains unchanged for 2 weeks. *No colonies were observed at the fourth passage for the two mice injected with 10^7 cells. (–□–, wild type; –◪–, heterozygous deficiency; –■–, homozygous deficiency; vertical bars, standard deviation of mean. The data symbols without error bars indicate that the deviation is within each symbol.)

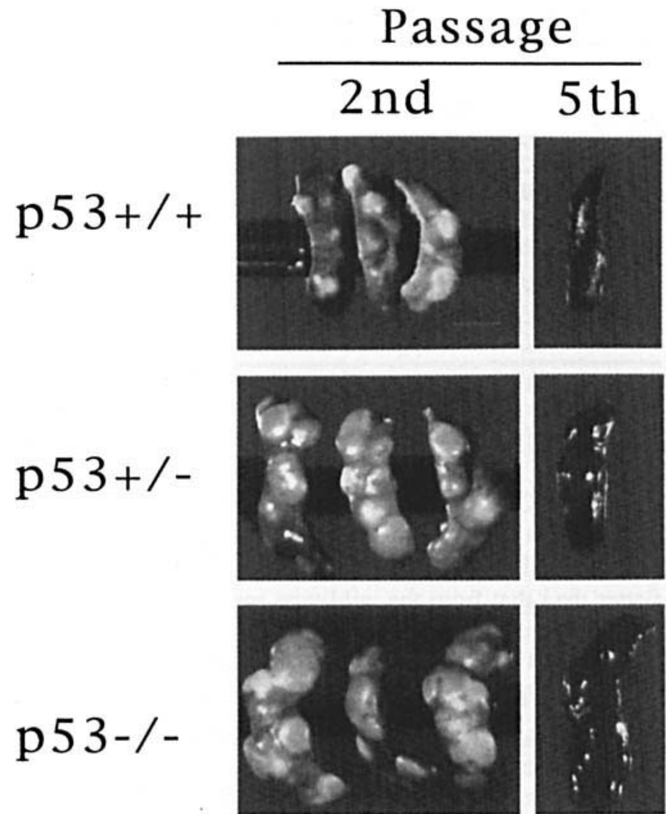

Figure 5. Spleen colony size was larger for recipients repopulated with p53-deficient cells than that for recipients with wild-type cells. Sample spleens in the second and fifth passages are shown. (Passage 2 spleens fixed in Bouin's solution; passage 5 spleens unfixed. Horizontal scale indicates 3 mm).

(Fig. 5, bottom panel) and heterozygous p53-deficient (Fig. 5, middle panel) BM cells. The difference was more prominent at passage 5 than at passage 2 (Table I).

Rapid Proliferation of p53-Deficient Hemopoietic Progenitors. In order to elucidate the mechanism underlying the long-term sustained growth of p53-deficient hemopoietic stem cells, cell kinetics in each compartment of three different hemopoietic progenitors was determined by the BUUV assay. In Figure 6, the cell kinetics of primitive hemopoietic progenitors (CFUS-13), i.e., spleen colonies observed on Day 13 (indicated by S13, Fig. 6, bottom panel), mature hemopoietic progenitors (CFU-S9), i.e., spleen colonies observed on Day 9 (indicated by S9, Fig. 6, middle panel), and the progenitors cultured with GM-CSF, i.e., CFU-GM (indicated by GM, Fig. 6, top panel) is shown. In this assay, the slopes reflect the population doubling of each progenitor fraction.

Wild-type progenitors, indicated by open symbols in Figure 6, grow exponentially up to the stationary phase within the first 2 to 5 days, depending on the progenitor hierarchy, with different slopes from S13 to GM. In general, primitive progenitors grow with a steeper slope, matured progenitors grow with a moderately steep slope, and cultured progenitors with a much flatter slope. The trend of this sequential change is essentially the same as that indicated by closed symbols in Figure 6. However, interestingly, the

Table I. Size of Spleen Colonies (CFU-S)

Passage number	Genotype	Diameter of colony mean ± SD (mm)	(Number of colonies examined)
2	Wild type	2.35 ± 0.78	(10)
	p53 heterozygous KO	2.85 ± 0.66	(17)
	p53 homozygous KO	3.00 ± 0.81[a]	(22)
5	Wild type	n.d.[b]	(0)
	p53 heterozygous KO	2.33 ± 0.76	(3)
	p53 homozygous KO	2.80 ± 0.76	(5)

[a] The average diameter of colonies was significantly larger than the wild type ($P < 0.05$).
[b] Not determined due to absence of visible colonies.

slopes for CFU-S9 and CFU-S13 from the p53 KO are steeper than those from the wild-type mice, whereas the slope for CFU-GM from the p53 KO mice is somewhat flatter than that from the wild-type mice.

Discussion

We have attempted to address the question of whether hemopoietic stem cells are infinitely mitotable. This is of clinical interest, not only with respect to cellular senescence, but also with respect to the quality of hemopoietic stem/progenitor cells before and after BMT.

In Siminovitch and coworkers' seminal study (9), 10^6 murine BM cells were transplanted into lethally irradiated recipients, and the resultant spleen cells or spleen colony-derived cells were passaged serially into secondary and tertiary recipients at 14-day intervals. The study was terminated after three passages when no further colony growth could be detected. This was probably because the stimuli to differentiate were maximized and dominated the stimuli for self-renewal.

In our present study, we attempted to determine whether more rapidly proliferating stem cells could be serially transplanted for a period longer than that achieved using Siminovitch and coworkers' protocol (9). We queried whether more rapidly proliferating hemopoietic stem cells could generate a larger progeny population from hemopoietic progenitor cells. For this purpose, we employed the BUUV method, which involved identifying the hemopoietic progenitor cells in the cell cycle by BrdUrd incorporation through an osmotic mini-pump, followed by purging those BrdUrd-incorporated cells specifically by exposure to UV light of a specific wavelength and determining the ratio of the number of hemopoietic colonies between the purged and the control cells (Fig. 6). As demonstrated by the BUUV assay (14, 15), hemopoietic stem cells from homozygous p53-KO mouse BM manifest an accelerated rate of proliferation under both steady-state and DNA-damage conditions.

Figures 2, 3, and 4 show that the number of successful serial passages varies inversely with the p53 gene dosage in the donor BM. Interestingly, colony growth did not cease

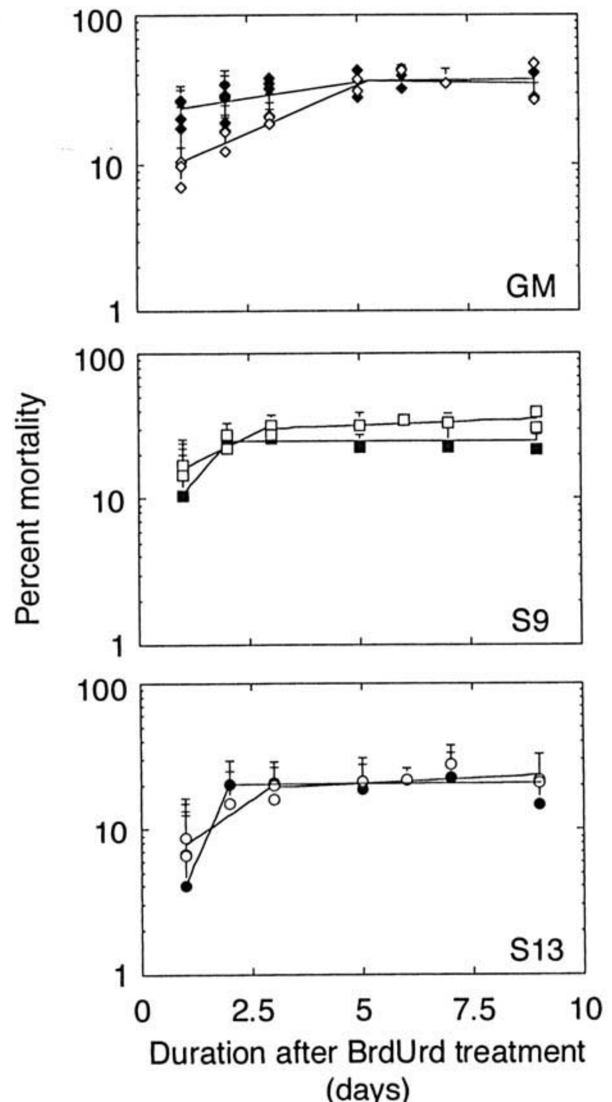

Figure 6. Cell kinetics of hemopoietic progenitor cells from the wild-type and homozygous p53-deficient mice was evaluated by the BUUV assay (see "Materials and Methods"). Upper panel shows kinetics of CFU-GM (diamond), middle panel shows kinetics of CFU-S-9 (square), and the bottom panel shows kinetics of CFU-S-13 (circle). Open symbols indicate the data for wild-type mice, and closed symbols indicate data for homozygous p53-deficient mice. All the factors of the regression lines (the slope, the Y intercept, and the r value) are tabulated in Table II.

immediately after cessation of logarithmic growth of the transplanted cells (Figs. 2 and 3). Moreover, the size and clonogenicity of the spleen colonies did not decrease after their growth potential decreased (Fig. 5 and Table I). The cessation of transplantability in Siminovitch and coworkers' protocol (9) was thought to be due to the loss of CFU-S. However, the present study indicates that failure of colony formation may have been due not to the absolute loss of CFUs-S, but rather to the decrease in the number of their immediate progenitors (LTRCs) to less than one per 10 CFUs-S (Fig. 4). Harrison and Zhong (16) have previously demonstrated significant differences between proliferative rates of long-term sustaining stem cells and multilineage

Table II. Doubling Time for Hemopoietic Progenitors

Progenitor cells	Genotype*1	(a)*2 Slope (percentage of mortality/days)	(b)*2 y intercept (%)	Doubling time*3 (hr)	r
CFU-GM	Wild	0.129	7.773	56.0	0.988
	p53 KO	0.042	21.95	172	0.970
CFU-S-9	Wild	0.149	11.29	48.5	0.936
	p53 KO	0.355	4.682	20.4	1.000
CFU-S-13	Wild	0.205	4.837	35.2	0.926
	p53 KO	0.708	0.784	10.2	1.000

*1) Genotype: Wild, wild type, p53 KO, p53 homozygous KO.
*2) Regression line: $y = b \times 10^{(ax)}$
x, duration after BrdUrd treatment (days)
y, percentage of mortality
a, cell cycle velocity (coefficient)
b, cycling ratio/unit time (coefficient)
*3) Doubling time (hr) = (log2/a) × 24

precursor cells. They noted that the latter tend to become extinct more rapidly than the former. Although we do not have data of the number of long-term sustaining stem cells, our data agree with those of our earlier study (17) indicating the probability that one out of every 10 CFCs is a long-term repopulating cell. In fact, long-term repopulating cells—which do not generate spleen colonies *per se* (18)—may have recovered during the 3-month or longer intervals between serial transplantations using the Harrison protocol (7).

Thus, the key to successful BMT is the quality of stem cells, the number of long-term repopulating stem cells, and the hemopoietic microenvironment (19). The development of technology for maintaining continuously proliferating long-term repopulating stem cells is essential for improving the efficiency of BMT. However, there is a information gap between the decrease in the number of hemopoietic stem cells and the induction of stem cell proliferation. Hence, there are some unresolved issues regarding stem cell proliferation following hemopoietic injury (20). BMT using p53-deficient-derived BM cells appears to be a good model for studies aimed at improving the methodology of BMT.

1. Cudkowicz G, Upton AC, Shearer GM, Highes WL. Limphocyte content and proliferative capacity of serially transplanted mouse bone marrow. Nature **201**:165–167, 1964.
2. Barnes DWH, Ford CE, Loutit JF. Greffes en serie de moelle osseuse homologue chez des souris irradiées. Sang **30**:762–765, 1959.
3. van Bekkum DW, Weyzen WEH. Serial transfer of isologous and homologous hematopoietic cells in irradiated hosts. Pathologie-Biologie **9**:888–893, 1961.
4. Barnes DWH, Loutit JR, Micklem HS. "Secondary disease" of radiation chimeras: a syndrome due to lymphoid aplasia. Ann NY Acad Sci **99**:374–385, 1962.
5. Koller PC, Doak SMA. Serial transplantation of haematopoietic tissue in irradiated hosts. In Harris RJC, Ed. Cellular Basis and Aetiology of Late Somatic Effects of Ionizing Radiation. London: Academic Press, pp59–64, 1963.
6. Harrison DE. Normal production of erythrocytes by mouse marrow continuous for 73 months. Proc Nat Acad Sci USA **70**:3184–3188, 1973.
7. Harrison DE. Mouse erythropoietic stem cell lines function normally 100 months: loss related to number of transplantations. Mech Ageing Dev **9**:427–433, 1979.
8. Chen J, Astle CM, Harrison DE. Genetic regulation of primitive hematopoietic stem cell senescence. Exp Hematol **28**:442–450, 2000.
9. Siminovitch L, Till JE, McCulloch EA. Decline in colony-forming ability of marrow cells subjected to serial transplantation into irradiated mice. J Cell Com Physiol **64**:23–32, 1964.
10. Tsukada T, Tomooka Y, Takai S, Ueda Y, Nishiwawa S-I, Yagi T, Tokunaga T, Takeda N, Suda Y, Abe S, Matsuo I, Ikawa Y, Aizawa S-I. Enhanced proliferative potential in culture of cells from p53-deficeint mice. Oncogene **8**:3313–3322, 1993.
11. Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res **14**:213–222, 1961.
12. Hirabayashi Y, Inoue T, Yoshida K, Inayama Y, Kanisawa M. The detection of normal hidden stem cells during the development of leukemia: assays with PGK isozyme. Exp Hematol **18**:7–10, 1990.
13. Tanaka T, Suda T, Suda J, Inoue T, Hirabayashi Y, Hirai H, Takaku F, Miura Y. Stimulatory effects of granulocyte colony-stimulating factor on colony-forming units-spleen (CFU-S) differentiation and pre-CFU-S proliferation in mice. Blood **77**:2597–2602, 1991.
14. Hirabayashi Y, Matsumura T, Matsuda M, Kuramoto K, Motoyoshi K, Yoshida K, Sasaki H, Inoue T. Cell kinetics of hemopoietic colony-forming units in spleen (CFU-S) in young and old mice. Mech Ageing Devel **101**:221–231, 1998.
15. Yoon BI, Hirabayashi Y, Kawasaki Y, Kodama Y, Kaneko T, Kim DY, Inoue T. Mechanism of action of benzene toxicity: cell cycle suppression in hemopoietic progenitor cells (CFU-GM). Exp Hematol **29(3)**:278–285, 2001.
16. Harrison DE, Zhong RK. The same exhaustible multilineage precursor produces both myeloid and lymphoid cells as early as 3–4 weeks after marrow transplantation. Proc Natl Acad Sci USA **89**:10134–10138, 1992.
17. Cronkite EP, Inoue T, Hirabayashi Y, Bullis J. Are stem cells exposed to ionizing radiation in vivo as effective as nonirradiated transfused stem cells in restoring hematopoiesis? Exp Hematol **21(6)**:823–825, 1993.
18. Morrison SJ, Weissman IL. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. Immunity **1(8)**:661–673, 1994.
19. Charbord P. Hemopoietic stem cells: analysis of some parameters critical for engraftment. Stem Cells **12**:545–562, 1994.
20. Robinson S, Van Os R, Sheridan T, Mauch P. Reduction of marrow hemopoietic progenitor and stem cell content is not sufficient for enhanced syngeneic engraftment. Stem Cells **18**:93–101, 2000.
21. Inoue T, Bullis JE, Cronkite EP, Hubner GE. Relationship between number of spleen colonies and 125IdUrd incorporation into spleen and femur. Proc Natl Acad Sci USA **80**:435–438, 1983.