Long-Term Monoclonal Reconstitution of Erythropoiesis in Genetically Anemic $W/W^*$ Mice by Injection of 5-Fluorouracil-Treated Bone Marrow Cells of $Pgtk-1^a/Pgtk-1^a$ Mice

By Toru Nakano, Noriko Waki, Hidekazu Asai, and Yukihiko Kitamura

The spleen colony-forming assay does not represent the number of hematopoietic stem cells with extensive self-maintaining capacity because five to 50 spleen colony-forming units (CFU-S) are necessary to rescue a genetically anemic (W/B X C57BL/6)F1-$W/W^*$ (WBB6F1-$W/W^*$) mouse. We investigated which is more important for the reconstitution of erythropoiesis, the transplantation of multiple CFU-S or that of a single stem cell with extensive self-maintaining potential. The electrophoretic pattern of hemoglobin was used as a marker of reconstitution and that of phosphoglycerate kinase (PGK), an X chromosome-linked enzyme, as a tool for estimating the number of stem cells. For this purpose, we developed the C57BL/6 congenic strain with the $Pgtk-1^a$ gene. Bone marrow cells were harvested after injection of 5-fluorouracil from C57BL/6-$Pgtk-1^a/Pgtk-1^a$ female mice in which each stem cell had either A-type PGK or B-type PGK due to the random inactivation of one of two X chromosomes. When a relatively small number of bone marrow cells (ie, $10^3$ or $3 \times 10^3$) were injected into 200-rad-irradiated WBB6F1-$W/W^*$ mice, the hemoglobin pattern changed from the recipient type ($Hbb'/Hbb'$) to the donor type ($Hbb'/Hbb'$) in seven of 150 mice for at least 8 weeks. Erythrocytes of all these WBB6F1-$W/W^*$ mice showed either A-type PGK alone or B-type PGK alone during the time of reconstitution, which suggests that a single stem cell with extensive self-maintaining potential may sustain the whole erythropoiesis of a mouse for at least 8 weeks.

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pattern changed from the recipient type (Hbb'/Hbb') to the donor type (Hbb'/Hbb) for at least 8 weeks in seven of 150 mice. Erythrocytes of all these seven WBB6F1-W/W' mice showed either A-type PGK alone or B-type PGK alone, thereby suggesting that a single stem cell with extensive self-maintaining capacity may sustain the whole erythropoiesis of a mouse at least for 8 weeks.

MATERIALS AND METHODS

Mice. C57BL/6-Pgk-1'Pgk-1' and WBB6F1-W/W' mice were raised in our laboratory. The original stock of mice with the Pgk-1' gene was introduced from Dr Vern M. Chapman, Roswell Park Memorial Institute, to the National Cancer Institute, Tokyo, by Dr Hiroshi Tanooka. We obtained C57BL/6-Pgk-1' gene from Dr Tanooka and introduced the Pgk-1' gene to the C57BL/6 strain of our inbred colony by repeated backcrosses (11 backcrosses at the time of the present study). Because the original C57BL/6 strain has the usual-type PGK gene (Pgk-1'), C57BL/6-Pgk-1'/Pgk-1' mice were produced by mating C57BL/6-Pgk-1'/Pgk-1' female mice with C57BL/6-Pgk-1'/Y male mice. Resulting C57BL/6-Pgk-1'/Pgk-1' mice were used as donors of bone marrow cells at 2 to 3 months of age. The A-type/B-type ratio of PGK in erythrocytes of C57BL/6-Pgk-1'/Pgk-1' mice is about 50/50.

Although the W' mutant gene was derived from the Jackson Laboratory, Bar Harbor, ME, it is now maintained in our inbred C57BL/6 mouse colony. The WB-W' + strain, which was also obtained from the Jackson Laboratory, has been maintained by brother-sister mating. WBB6F1-W/W' mice that had been produced by a cross between WB-W'/+ and C57BL/6-W'/+ mice, were used as recipients at 4 months of age.

Hemoglobin of WBB6F1-W/W' mice (Hbb'/Hbb) can be distinguished from that of C57BL/6-Pgk-1'/Pgk-1' mice (Hbb'/Hbb') by electrophoresis.20,21

5FU treatment and bone marrow transplantation. 5FU (150 μg/g body weight) was injected intravenously to C57BL/6-Pgk-1'/Pgk-1' mice; the mice were killed four days after the 5FU injection, and bone marrow cells were collected from at least five mice and suspended in Eagle's medium according to the method described previously.12

Recipient WBB6F1-W/W' mice were irradiated (200 rad) with a Shimadzu x-ray machine (180 kV and 20 mA, with a 2-mm Al filter, 50 rad/min) to facilitate the bone marrow reconstitution. Various numbers of bone marrow cells were injected through the lateral tail vein.

Assay of CFU-S. The method of Till and McCulloch22 was used; the recipient WBB6F1-W/W' mice were killed eight or 14 days after the transplantation of bone marrow cells; spleens were removed and fixed in Bouin's solution, and colonies were counted with a dissection microscope (7x).

Sampling of blood. Mice were anesthetized with ether, and blood samples were obtained from the retroorbital sinus with heparinized microhematocrit tubes. Care was taken to remove small volumes (<50 μL) of blood to minimize possible hematologic stress. The microhematocrit tube was centrifuged, and the tube was cut beneath the level of the buffy coat; packed erythrocytes were divided into two parts; one part was used for examination of hemoglobin and another part for examination of PGK.

Examination of hemoglobin. Electrophoresis of hemoglobin was done after modification with cystamine according to the method described by Whitney.23 The hemoglobin pattern was determined visually by using known mixtures as standards. By this method, hemoglobin of WBB6F1-W/W' mice (Hbb'/Hbb) mixed with hemoglobin of C57BL/6-Pgk-1'/Pgk-1' mice (Hbb'/Hbb) is detectable if the former contributes ±5% of the total. We assumed that WBB6F1-W/W' recipient mice were reconstituted by bone marrow cells of C57BL/6-Pgk-1'/Pgk-1' mice only when the hemoglobin pattern of the former could not be distinguished from that of the latter. In other words, >95% of erythrocytes in the reconstituted WBB6F1-W/W' mice were derived from the bone marrow of C57BL/6-Pgk-1'/Pgk-1' mice.

Electrophoresis of PGK. Small pieces of filter paper were soaked with blood samples and placed in small plastic tubes; the tubes were kept at −80°C. The PGK pattern of erythrocytes was examined only when WBB6F1-W/W' recipient mice were assumed to be reconstituted by bone marrow cells of C57BL/6-Pgk-1'/Pgk-1' mice. After thawing, pieces of filter paper with blood samples were inserted into the gel plate. The electrophoresis was carried out on a gel plate (10 × 20 cm) of starch (Wako Chemical Co, Tokyo) for 17 hours at 5 V/cm and 4°C according to the method described by Tanooka and Tanaka.24 The gel plates were then cut into two slices and PGK was detected as nonfluorescent spots due to conversion of NADH to NAD in the PGK assay system. The spot of A-type PGK was usually 6 cm from the origin, and that of B-type was 4 cm from the origin. According to the report of Reddy and Fialkow,25 the relative activity of PGK alloanzymes was estimated visually by using known mixtures as standards. With this technique, a minor population in a mixture of blood is detectable if it contributes ±5% of the total PGK activity.

The proportion of A-type PGK in serial blood samples was plotted as the deviation of individual samples from the percentage of A-type PGK in C57BL/6-Pgk-1'/Pgk-1' mice according to Burton et al.12 Therefore, +50% indicated that only A-type PGK was present, 0% that A-type PGK and B-type PGK were equally mixed, and −50% that only B-type PGK was present.

Limiting-dilution analysis. The principle of the method has been described by Porter and Berry,26 Breivik,27 and Boggs et al,28 and briefly it was carried out as follows. Random samples from a homogeneous cell population were assayed for the presence or absence of stem cells. Such cells should be distributed among samples in a Poisson fashion. The finite probability that a sample will not contain a stem cell is defined by $P_0 = e^{-\phi}$ where $\phi$ is the fraction of stem cells in the sample of x cells. $P$ is the proportion of samples devoid of stem cells and estimates $P$. The concentration of stem cells in the sample can then be expressed as $\phi = -\ln P/x$.

RESULTS

First, we confirmed the effect of 5FU treatment in the present experimental condition; bone marrow cells from 5FU-treated and control C57BL/6-Pgk-1'/Pgk-1' mice were injected into 200-rad-irradiated WBB6F1-W/W' mice. The recipient mice were divided into two groups; one group of mice was killed on day 8 and the other group on day 14. The 5FU treatment decreased the number of bone marrow cells from about 25 × 10⁶/femur to about 2 × 10⁶/femur but increased the day 14 to day 8 ratio of spleen colonies as reported by Hodgson and Bradley29 and van Zant30 (Table 1). Because hematopoietic stem cells that form day 14 colonies are considered to have more extensive proliferative potential than those that form day 8 colonies,6 we used bone marrow cells of 5FU-treated mice throughout the present study.

Because we injected as few as 10⁵ 5FU-treated bone marrow cells in the next experiment, we confirmed the number of CFU-S in such a small number of 5FU-treated bone marrow cells of C57BL/6-Pgk-1'/Pgk-1' mice. As shown in Table 1, 10 × 10⁵ cells contained ~2.2 day 14 CFU-S and 2 × 10⁵ cells, ~0.4 day 14 CFU-S.
Various numbers of bone marrow cells from 5FU-treated C57BL/6-Pgk-1/p/Pgk-1 mice were injected into 200-rad-irradiated WBB6F1-W/W mice; the hemoglobin pattern of the recipients was examined at 4-week intervals. When the hemoglobin pattern of WBB6F1-W/W recipient mice (Hbb2/Hbb2) changed to pure Hbb1/Hbb1, the PGK pattern of erythrocytes was also examined.

In total, 303 WBB6F1-W/W mice survived at least 16 weeks after bone marrow transplantation; in eight WBB6F1-W/W mice, a pure Hbb2/Hbb2 hemoglobin pattern was detected only at one observation time (transient reconstitution in Table 2, Fig 1), whereas pure Hbb1/Hbb1 pattern was maintained at least during 8 weeks (ie, three observation times) in 48 WBB6F1-W/W mice (long-term reconstitution in Table 2, Fig 2).

In 8 WBB6F1-W/W recipient mice that showed transient reconstitution, erythrocytes of five mice showed either A-type PGK or B-type PGK, whereas erythrocytes of the remaining three mice showed AB-type PGK throughout the time of transient reconstitution. When transient reconstitution was lost (ie, hemoglobin pattern returned to Hbb2/Hbb2), the PGK pattern of erythrocytes also returned to type B in all aforementioned eight WBB6F1-W/W mice.

WBB6F1-W/W mice that were reconstituted by bone marrow cells of C57BL/6-Pgk-1/p/Pgk-1 mice for a long term were divided into three groups by using the PGK pattern of erythrocytes as a criterion. In group 1, erythrocytes showed either A- or B-type PGK throughout the time of reconstitution. The reconstitution continued until the end of the observation period (40 weeks) or death in six of seven mice that belonged to group 1 (Fig 2A). In the remaining one WBB6F1-W/W mouse, the hemoglobin pattern came back to Hbb2/Hbb2 28 weeks after bone marrow transplantation (Fig 2B). In group 2, erythrocytes showed AB-type PGK when the change of hemoglobin pattern to pure Hbb1/Hbb1 was first detected. Then, the PGK pattern changed to either type A or B (Fig 2C). In group 3, erythrocytes showed AB-type PGK throughout the time of reconstitution (Fig 2D). In some mice of group 3, the type A-to-type B ratio of PGK was not always constant but fluctuated from time to time (Fig 2D). The distribution of WBB6F1-W/W recipient mice among the aforementioned three groups was apparently influenced by the number of injected bone marrow cells (Table 3). All WBB6F1-W/W mice that received 10^3 or 10^4 bone marrow cells from 5FU-treated C57BL/6-Pgk-1/p/Pgk-1 mice belonged to group 1, whereas all WBB6F1-W/W mice that received ≥50 × 10^3 cells belonged to groups 2 or 3.

We calculated the concentration of stem cells that may reconstitute the whole erythropoiesis of WBB6F1-W/W mice for a long term by using limiting-dilution analysis. From data shown in Table 2, we calculated the proportion of

Table 1. Numbers of Day 8 and Day 14 Spleen Colonies in 200-rad-Irradiated WBB6F1-W/W Mice After Injection of 5FU-Treated and Nontreated Bone Marrow Cells

<table>
<thead>
<tr>
<th>5FU Treatment*</th>
<th>No. of Cells Injected (x 10^3)</th>
<th>No. of Colonies (Mean ± SE)†</th>
<th>Ratio of Day 14/Day 8 Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 8</td>
<td>Day 14</td>
</tr>
<tr>
<td>No</td>
<td>50</td>
<td>4.4 ± 1.7 (10)</td>
<td>4.8 ± 1.0 (13)</td>
</tr>
<tr>
<td>Yes</td>
<td>50</td>
<td>0.7 ± 0.3 (12)</td>
<td>12.6 ± 0.8 (16)</td>
</tr>
<tr>
<td>Yes</td>
<td>10</td>
<td>ND</td>
<td>2.2 ± 0.6 (11)</td>
</tr>
<tr>
<td>Yes</td>
<td>2</td>
<td>ND</td>
<td>0.4 ± 0.1 (12)</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.

*Bone marrow cells were obtained from C57BL/6-Pgk-1/p/Pgk-1 mice that had been injected with 5FU (150 μg/g body weight) four days before sacrifice.

†The number of recipients is shown in parenthesis.

Fig 1. Typical cases of transient reconstitution. Electrophoretic pattern of hemoglobin of WBB6F1-W/W mouse (Hbb1/Hbb1) changed to the donor type (Hbb1/Hbb1) at 4 weeks (A) or 8 weeks (B) after the injection of bone marrow cells from 5FU-treated C57BL/6-Pgk-1/p/Pgk-1 mice; 10 × 10^3 cells were injected in A and 3 × 10^2 cells in B.

Table 2. Number of WBB6F1-W/W Mice (Hbb1/Hbb1) in Which a Transient or Long-term Change of Hemoglobin Pattern Was Observed After Injection of Various Numbers of Bone Marrow Cells From 5FU-Treated C57BL/6-Pgk-1/p/Pgk-1 Mice (Hbb1/Hbb1)

<table>
<thead>
<tr>
<th>No. of Cells (x 10^3)</th>
<th>No. of Mice (Hbb1/Hbb1)</th>
<th>No. of Mice in Which Hemoglobin Pattern Changed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transient</td>
<td>Long-term</td>
</tr>
<tr>
<td>1</td>
<td>65</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3</td>
<td>85</td>
<td>3 (5)</td>
</tr>
<tr>
<td>10</td>
<td>49</td>
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</tr>
<tr>
<td>30</td>
<td>22</td>
<td>1 (5)</td>
</tr>
<tr>
<td>50</td>
<td>31</td>
<td>2 (6)</td>
</tr>
<tr>
<td>75</td>
<td>30</td>
<td>0 (0)</td>
</tr>
<tr>
<td>100</td>
<td>21</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Fig 1. Typical cases of transient reconstitution. Electrophoretic pattern of hemoglobin of WBB6F1-W/W mouse (Hbb1/Hbb1) changed to the donor type (Hbb1/Hbb1) at 4 weeks (A) or 8 weeks (B) after the injection of bone marrow cells from 5FU-treated C57BL/6-Pgk-1/p/Pgk-1 mice; 10 × 10^3 cells were injected in A and 3 × 10^2 cells in B.
Fig 2. Typical cases of long-term reconstitution. Proportions of donor-type hemoglobin and A-type PGK are shown. The data of the proportion of A-type PGK for each mouse are plotted as the deviation of individual samples from the percentage of A-type PGK in C57BL/6-Pgk-1<sup>+/</sup>/Pgk-1<sup>-</sup> mice according to Burton et al. Therefore, ± 50% indicates that only A-type PGK is present, 0% that A-type PGK and B-type PGK were equally mixed, and -50% that only B-type PGK is present. (A) The WBB6F<sub>1</sub>-<sub>W</sub>/<sub>W</sub> mouse was injected with 10<sup>6</sup> bone marrow cells from 5FU-treated C57BL/6-Pgk-1<sup>+/</sup>/Pgk-1<sup>-</sup> mice, erythrocytes showed B-type PGK alone throughout the observation periods (group 1). (B) The reconstitution ended at 28 weeks after the injection of 3 × 10<sup>5</sup> cells (group 1). (C) Erythrocytes showed both A-type and B-type PGK when hemoglobin first changed to the donor type at 8 weeks after the injection of 75 × 10<sup>6</sup> bone marrow cells. The PGK pattern changed to type A (group 2). (D) The WBB6F<sub>1</sub>-<sub>W</sub>/<sub>W</sub> mouse received 50 × 10<sup>6</sup> bone marrow cells. Although erythrocytes showed both A-type and B-type PGK throughout the observation period, the proportion of A-type PGK fluctuated gradually (group 3).

WBB6F<sub>1</sub>-<sub>W</sub>/<sub>W</sub> mice in which long-term complete reconstitution of erythropoiesis did not occur after bone marrow transplantation (proportion of unsuccessful transplantation), and plotted this against the number of injected cells (Fig 3). A straight line was obtained (P < .01 by χ<sup>2</sup> test) that intercepted the ordinate at 0.96. Therefore, the proportion of unsuccessful transplantations may be used to estimate the fraction of stem cells in 5FU-treated bone marrow cells. The slope of the line in Fig 3 is the estimate of the fraction of stem cells. Calculation of its 95% confidence limits was carried out according to the method described by Porter and Berry and Breivik.

DISCUSSION

When bone marrow cells of 5FU-treated C57BL/6-Pgk-1<sup>+/</sup>/Pgk-1<sup>-</sup> mice were injected into 200-rad-irradiated WBB6F<sub>1</sub>-<sub>W</sub>/<sub>W</sub> mice, the donor-type hemoglobin pattern (Hbb<sub>D</sub>/Hbb<sub>B</sub>) was detectable either transiently or for a long term (at least 8 weeks). Although the proportion of WBB6F<sub>1</sub>-<sub>W</sub>/<sub>W</sub> mice with the long-term reconstitution increased in parallel with the number of injected bone marrow cells, transient reconstitution did not occur when ∼75 × 10<sup>6</sup> bone marrow cells were injected. Transient reconstitution probably results from lodgment and differentiation of stem cell(s) with limited proliferative potential, whereas the long-term reconstitution may be attributable to the successful transplantation of stem cell(s) with extensive proliferative potential. When comparably large numbers of bone marrow cells were injected, the probability that both types of stem cells were successfully transplanted might increase. In such cases, transient and long-term reconstituions occurred at the same time and were recognized as long-term reconstitution.

The PGK pattern of erythrocytes in long-term reconstituted WBB6F<sub>1</sub>-<sub>W</sub>/<sub>W</sub> mice was influenced by the number of injected bone marrow cells. The proportion of reconstituted mice with double enzyme patterns increased in parallel with the number of cells. When each WBB6F<sub>1</sub>-<sub>W</sub>/<sub>W</sub> mouse received 10<sup>4</sup> or 3 × 10<sup>5</sup> bone marrow cells, erythrocytes of all seven long-term reconstituted WBB6F<sub>1</sub>-<sub>W</sub>/<sub>W</sub> mice showed either an A-type or B-type PGK pattern (group 1 of Fig 2). The result appears to indicate that a single stem cell with

<table>
<thead>
<tr>
<th>No. of Bone Marrow Cells Injected (x 10&lt;sup&gt;6&lt;/sup&gt;)</th>
<th>No. of Mice at the Following PGK Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

This is the same experiment as shown in Table 2.
extensive proliferative potential reconstitutes the whole erythropoiesis of these seven WBB6F-/-W/W' mice for at least 8 weeks due to the following reason. In a population of stem cells consisting of equal numbers of A- and B-type cells, the probability that proliferation of n cells occurs in A-type cells only or in B-type cells only is \((1/2^n) \times 2\), ie, \(1/2^{n-1}\). Consequently, the 100% (7/7) incidence of single-enzyme pattern cells only or in B-type cells only is \((1/2) \times 2\), ie, \(1/2\) the probability that proliferation of stem cells are injected into 150 WBB6F-/-W/W' mice, the possibility of monoclonal reconstitution cannot be explained in the case of \(n \geq 2\).

Estimation by limiting-dilution analysis also supports the possibility of monoclonal reconstitution. Because \(10^5\) bone marrow cells were injected into 65 WBB6F-/-W/W' mice and \(3 \times 10^3\) cells into 85 WBB6F-/-W/W' mice, in total, \(3.2 \times 10^3\) cells were injected into 150 WBB6F-/-W/W' mice. The concentration of stem cells with an extensive proliferative potential was \(7.7 \times 10^3\) bone marrow cells, which indicated \(\sim 5\) such stem cells in \(3.2 \times 10^3\) bone marrow cells. When \(\sim 5\) stem cells are injected into 150 WBB6F-/-W/W' mice, the possibility that two stem cells are injected into a single WBB6F,/-W/W' mouse should be low.

When calculated by using the concentration of day 14 CFU-S shown in Table 1, \(3.2 \times 10^3\) bone marrow cells from 5FU-treated C57BL/6-Pgk-/-/Pgk-/- mice have the capacity to produce about 60 to 81 day 14 spleen colonies in 200-rad-irradiated WBB6F-/-W/W' mice. If a single day 14 CFU-S had been able to reconstitute the whole erythropoiesis of a WBB6F-/-W/W' mouse, about a half of 150 WBB6F-/-W/W' mice injected with \(10^5\) or \(3 \times 10^3\) bone marrow cells would have shown a pure Hbb'/Hbb' hemoglobin pattern. In fact, three WBB6F-/-W/W' mice showed a pure Hbb'/Hbb' pattern transiently and seven WBB6F,/-W/W' mice for a long term. Therefore, most of the stem cells that produce a day 14 spleen colony do not appear to have the potential to reconstitute the whole erythropoiesis of a WBB6F,/-W/W' mouse even transiently. This is consistent with the results of Wiktor-Jedrzejczak et al,8 Boggs et al,9 and Visser et al,27 that the CFU-S assay does not represent the number of stem cells with extensive proliferative potential.

Wiktor-Jedrzejczak et al,8 Boggs et al,9 and Visser et al,27 used bone marrow cells from nontreated syngeneic donors, whereas we harvested bone marrow cells from 5FU-treated donors of the parental strain origin. The number of bone marrow cells necessary to reconstitute a WBB6F-/-W/W' mouse was smaller in their experiments than in our present experiment. Although 5FU treatment significantly increased the day 14–to-day 8 ratio of colonies, it does not appear to increase the proportion of stem cells with extensive proliferative potential. However, because we used C57BL/6-Pgk-l'/Pgk-l' mice rather than syngeneic +/+ mice as donors, there is a possibility that the hybrid resistance reported by Harrison et al,28 in the present strain combination may reduce the efficiency of the stem cells of C57BL/6 strain origin.

Long-term reconstitution occurred in 33 of 82 WBB6F-/-W/W' mice after transplantation of \(50 \times 10^3\) to \(100 \times 10^3\) bone marrow cells from 5FU-treated C57BL/6-Pgk-l'/Pgk-l' mice. In all these 33 WBB6F-/-W/W' mice, the PGK pattern of erythrocytes was of the AB type when the hemoglobin pattern first changed to the Hbb'/Hbb' type. The PGK pattern changed from a double-enzyme pattern to a single-enzyme pattern in 16 of 33 WBB6F-/-W/W' mice (group 2 of Fig 2), but the double-enzyme pattern remained in 17 WBB6F-/-W/W' mice (group 3 of Fig 2).

There are two possible explanations for group 2. (a) The reconstitution may result from the lodgment and differentiation of both a stem cell(s) with limited proliferative potential and a single stem cell with extensive proliferative potential. A stem cell with extensive proliferative potential continued the proliferation and differentiation after the expiration of stem cell(s) with limited proliferative potential. (b) The reconstitution may be initiated by lodgment and differentiation of two or more stem cells with extensive proliferative potential. However, subsequent emergence of a single monoclonal population occurred. Probably, stem cells belonging to the other clone(s) may remain in the hematopoietic tissues, but their differentiation was suppressed by unknown mechanisms. Determination of PGK patterns of CFU-S and in vitro colony-forming cells from such hematopoietic tissues may prove or disprove the possibilities.

In group 3, long-term reconstitution appears to be attributable to the lodgment of \(\geq 2\) stem cells with extensive proliferative potential. Even when the AB-type PGK pattern remained throughout the observation period, the type A-to-type B PGK ratio fluctuated in some WBB6F-/-W/W' recipients. This is consistent with the recent result of Lemischka et al,28 and may be explained by the concept of clonal succession proposed by Kay.29

To investigate the fate of a single stem cell, Abramson et al,30 induced chromosomal aberrations by x-irradiation, and Dick et al,31 Lemischka et al,28 and Keller et al,32 marked the stem cells with the integration sites of retrovirus. These markers are very useful because of the huge numbers of variations. However, when these markers are used, it seems difficult to exclude the influences of irradiation or retrovirus infection. Moreover, clones that failed to bear abnormal chromosomes or retrovirus and clones of the recipient origin are not detectable. These difficulties may be overcome with the present system in which electrophoretic patterns of hemoglobin and PGK are used as markers. Furthermore, vast numbers of recipient mice can be investigated frequently because it is much easier to examine patterns of hemoglobin and PGK than to examine chromosomal aberrations or integration sites of retrovirus. The present system is especially useful as a model of monoclonal reconstitution because a lot of information about human stem cell disorders such as CML, polycythemia vera, and agnogenic myeloid metaphasia has been accumulated by using another X chromosome-linked enzyme, G6PD, as a marker.10

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REFERENCES


Long-term monoclonal reconstitution of erythropoiesis in genetically anemic W/Wv mice by injection of 5-fluorouracil-treated bone marrow cells of Pgk-1b/Pgk-1a mice

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