CONCISE REPORT

Normal Cycling Patterns of Hematopoietic Stem Cell Subpopulations: An Assay Using Long-Term In Vivo BrdU Infusion

By M.E. Pietrzyk, G.V. Priestley, and N.S. Wolf

It was found in a long-term bromodeoxyuridine (BrdU) infusion study that two or more different subpopulations of bone marrow stem cells exist in mice. One of these subpopulations appears to be noncycling and forms approximately 10% of eight-day CFU-S. Another one, a subpopulation of slowly cycling bone marrow cells, is represented as 14-day CFU-S. The 14-day CFU-S have a regular increment in the percentage of the subpopulation entering the cycle over time, with a cell generation half-time of 21 days. The cycling status in these experiments was ascertained by in vivo continuous long-term BrdU infusion. An improved method is presented for long-term BrdU infusion with UV killing of cycled cells.

MATERIALS AND METHODS

Fifteen adult male bone marrow donor mice of strain (C57B1/6 × DBA/2)F1 (BDF1) were implanted subcutaneously at dorsal thoracic site with two mini osmotic pumps (model no. 2001 Alza Corp., Palo Alto, Calif) each loaded with sufficient BrdU to provide continuous infusion at the rate of 2 mg/kg/h for ten days. BrdU solutions were prepared in sterile 1% ammonia water solution, an important factor in allowing complete dissolving of the compound without heating. Pump loading and start-up conditions were according to manufacturer’s recommended procedures. Pumps were replaced at the seventh to ninth day under light anesthesia. On designated days three donors were killed, and their femurs were flushed with Hanks’ buffered saline solution according to our previously described method and under conditions preventing exposure to daylight or to fluorescent light (this light-excluding condition was maintained throughout the entire procedure of handling bone marrow cells [BMC] including the injection). Single-cell suspensions were counted for the number of viable cells and diluted to 10^6 or 10^7 BMC per mL. Part of this suspension was left without UV treatment and injected into equal numbers of male and female syngeneic, lethally irradiated (1,200 to 1,250 rad from a ^31CS source, ~50 rad/min) control recipients at 5 x 10^5 BMC each. The major part of the suspension was exposed to 150 ergs of UV radiation (single mercuric lamp with 85% spectrum in a 254-nm wavelength at a distance of 88 cm for 12 seconds in a cell suspension depth of 2 mm at a concentration of 10^6 or 10^7 cells per mL) aliquoted for different dose levels and injected intravenously (IV) into irradiated experimental groups (12 to 24 animals per group). Animals were killed on the days indicated; spleens were removed, fixed in Bouin’s solution, and the colonies counted at 10x magnification. Irradiation control mice showed no colonies in the spleen.

RESULTS

The results are shown in Fig 1 and Table 1. The assay for early appearing colonies showed a mean of approximately 90% UV killing at each time point as compared with the eight-day sacrifice, non-UV-irradiated control recipients. The late CFU-S kill (cycling) pattern results showed an accumulation rate of killed (cycled) cells of 2.58%/d with 50% of that population having passed through S phase by day 21. Through the 32nd day in the non-UV-treated groups there was no reduction in either the eight-day or 14-day...
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Fig 1. Normal cycling patterns of CFU-S subpopulations as revealed by BrdU incorporation and UV killing. Early CFU-S are those assayed at eight days. For infusion days 7 through 32, the slope is 0.10 and the linear correlation coefficient (r) is 0.68. Late CFU-S are those assayed at 14 days. For infusion days 7 through 32, the slope is 2.58 and the linear correlation coefficient (r) is 0.99. The percentage killed was calculated from a difference in the means of UV-treated vs non-UV-treated groups corrected for cell dose. The control group BMC dose was 5 x 10⁶ at each point. UV BMC doses were adjusted to give readable colony numbers in recipient spleens at each kill point.

Inconsistent results in our own early experiments as well as conflicting reports from other laboratories demonstrate a need for long-term, continuous infusion for observation of the patterns of CFU kinetics. Our findings clarify those of Patt et al and of Hagan and MacVittie. The former suggested a cell cycle half-time of six days and the latter, a mean time of 52 hours for nearly all CFU-S measured. These investigators examined only the early CFU-S (less than 11 day posttransplantation). We also note a difference for seven-day BrdU infusion between our data for late CFU-S survival (Fig 1) and that of Hodgson et al. Our data suggest that a part of this CFU-S subpopulation has a cycle time longer than 32 days.

We understand that a number of questions still remain to be answered: when, if at all, the entire late CFU-S subpopulation will cycle and whether the constant 10% of noncycling

colony number from that found in normal untreated bone marrow (Table 1). Also, controls run with 150 ergs of UV irradiation of normal BMC in a different set of experiments showed no reduction in either early or late colony numbers (data not shown). Four preliminary experiments (before the method was perfected) with a 17-day BrdU infusion using different mouse strains showed results consistent with the data presented here.

DISCUSSION

It has been reported previously that the early and late CFU-S come (largely) from different stem cell populations. Our data for cycling patterns of normal, non-stressed hematopoietic stem cell subpopulations reconfirms and strongly supports such conclusions and begins to elucidate the dynamics of these subpopulations of CFU-S.

Table 1. Colony Numbers at Eight and 14 Days in Recipients of Bone Marrow Treated with BrdU ± UV

<table>
<thead>
<tr>
<th>Number of Days</th>
<th>Day 8 Sacrifice</th>
<th>Day 14 Sacrifice</th>
</tr>
</thead>
<tbody>
<tr>
<td>BrdU Inf</td>
<td>+ UV</td>
<td>No UV</td>
</tr>
<tr>
<td>0</td>
<td>ND</td>
<td>10.83 (±0.56)</td>
</tr>
<tr>
<td>7</td>
<td>1.13 (±0.18)</td>
<td>10.83 (±1.36)</td>
</tr>
<tr>
<td></td>
<td>N = 15</td>
<td>N = 12</td>
</tr>
<tr>
<td>14</td>
<td>1.1 (±0.075)</td>
<td>10.17 (±0.89)</td>
</tr>
<tr>
<td></td>
<td>N = 15</td>
<td>N = 12</td>
</tr>
<tr>
<td>21</td>
<td>1.12 (±0.07)</td>
<td>9.92 (±1.02)</td>
</tr>
<tr>
<td></td>
<td>N = 15</td>
<td>N = 12</td>
</tr>
<tr>
<td>28</td>
<td>0.85 (±0.05)</td>
<td>7.75 (±0.49)</td>
</tr>
<tr>
<td></td>
<td>N = 15</td>
<td>N = 12</td>
</tr>
<tr>
<td>32</td>
<td>0.75 (±0.04)</td>
<td>10.33 (±0.77)</td>
</tr>
<tr>
<td></td>
<td>N = 18</td>
<td>N = 18</td>
</tr>
</tbody>
</table>

Abbreviations: N, number of mice surviving to kill; ND, not determined.
*Normal bone marrow recipients killed on days 8 and 12.
Mean spleen colony number (±SE)/5 x 10⁶ BMC is shown.
early CFU-S is a real event maintained over a long period of time. We also understand that cycling patterns do not fully characterize the early and late spleen colony-forming cell populations. However, the results presented here document the presence of CFU-S subpopulations with greatly different cell cycle intervals and suggest that, at least for eight-day CFU-S, the differences in this characteristic may not always coincide with the time of appearance of the spleen colonies derived from these subpopulations.

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REFERENCES

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