The Repopulation Potential of Fetal Liver Hematopoietic Stem Cells in Mice Exceeds That of Their Adult Bone Marrow Counterparts

By Vivienne I. Rebel, Cindy L. Miller, Connie J. Eaves, and Peter M. Lansdorp

Varying, limiting numbers of unseparated or purified cells (Ly-5.1), either from 14.5-day-old fetal liver (FL) or from adult bone marrow (BM) were co-injected with 10^5 unseparated BM cells (Ly-5.2) into lethally irradiated adult C57B1/6 recipients (Ly-5.2). The kinetics of donor cell repopulation of the lymphoid and myeloid compartments by Ly-5.1+ donor hematopoietic stem cells (ie, competitive repopulation units [CRU]) were monitored at various time points after the transplantation by Ly-5 analysis of the peripheral white blood cells (WBC). Recipients that had received on average less than 2 adult BM or FL CRU did not show a significant difference in the level of donor-reconstitution when analyzed 4 weeks after the transplantation. However, at 8 and 16 weeks, the FL recipients showed a significantly higher percentage of donor-derived nucleated peripheral blood cells than did the recipients of adult BM cells. Analysis of individual mice showed that approximately 80% of the recipients of FL CRU showed an increase in mature WBC output between 4 and 8 weeks after transplantation, whereas this occurred in less than 40% in the recipients of adult BM cells. In addition to this effect on mature cell output, the cellularity of the reconstituted BM was significantly higher in recipients of FL CRU than in recipients of adult BM CRU, even at 7 to 9 months after transplantation, which is consistent with an increased clonal expansion of FL CRU. When marrow cells from primary recipients of FL CRU were injected into secondary recipients, a significantly higher percentage of these mice showed donor-reconstitution of their lymphoid and myeloid compartments (P < .01) and to a greater extent (P < .008) as compared with mice that had received marrow cells from primary recipients of similar numbers of adult BM CRU. Taken together, these results show that individual FL CRU exhibit a greater proliferative activity in vivo than similar cells from adult BM that is accompanied by a greater production of daughter CRU.

© 1996 by The American Society of Hematology.
MATERIALS AND METHODS

Animals. C57B1/6 (Ly-5.2) and C57B1/6 Ly-5.1:Pep3b (Ly-5.1) were bred and maintained in the animal facility of the British Columbia Cancer Research Center (Vancouver, British Columbia, Canada). All animals were kept under micro-isolators and were provided with sterilized food and acidified water (pH = 3) ad libitum.

Reconstitution experiments. C57B1/6 (Ly-5.2) mice (3 to 6 months old) were used as recipients. Whole body irradiation was administered in a single dose (110 cGy/min, 950 cGy in total) using a 60Co γ-ray source. All transplantations were performed within 1 to 6 hours after the irradiation. Donor adult BM cells were obtained from C57B1/6:Pep3b (Ly-5.1) mice and donor FL cells were obtained from 14.5-day-old fetuses that were of a hybrid Ly-5.2/Ly-5.1 phenotype. (At the day that the vaginal plug test result was positive, the gestational age of the fetuses was considered to be 0.5 days.) All primary recipients were cotransplanted with 10^7 normal adult (Ly-5.2) BM cells to assure the survival of the recipients. Secondary recipients were not additionally injected with such cells.

Contribution to the hematopoietic reconstitution by the test cell population was determined using an antigen-specific anti–Ly-5.1 monoclonal antibody (MoAb) as described previously.17,18 For this purpose, peripheral blood was obtained by tail-vein puncture and the nucleated cells were stained with the anti–Ly-5.1 MoAb. Recipients were considered to be multilineage repopulated ("positive") when the Ly-5.1+ cells represented greater than 1% of all the nucleated peripheral blood cells and included both myeloid and lymphoid cells as determined by their respective distinct side scatter properties.

Primary recipients. Primary recipients were transplanted with purified or unseparated cells of either FL or adult BM origin. The procedure used to obtain the purified subpopulations was extensively described elsewhere.17,18 The main difference between purification protocols for FL and adult BM CRU consisted of a larger orthogonal side scatter window for the selection of FL CRU and a cocktail of mature lineage markers that included B220, Gr-1, Ly-1, and Ter119 for FL cell suspensions (designated as "Lin−" in Table 1) and B220, Gr-1, Ly-1, and Mac-1 for adult BM cell suspensions (designated as "Lin+" in Table 2). CRU frequencies for every type of cell population used were determined by analysis of the proportion of negative mice in groups receiving limiting numbers of test cells, using Poisson statistics.19 Based on these frequencies, the average number of CRU received by a group of transplanted animals was calculated as follows: the number of cells transplanted divided by the frequency of CRU in the same subpopulation. Recipients of similar numbers of CRU from the same source, ie, either fetal liver or adult bone marrow were pooled, creating the following groups: animals that had received on average less than 0.5 CRU, between 0.5 and 1 CRU, between 1 and 2 CRU, and between 2 and 5 CRU and a group of mice that had received more than 5 CRU. Donor hematopoietic reconstitution was determined 4, 8, and 16 weeks after the transplantation.

Secondary recipients. In some experiments, BM cells isolated from both femurs and tibiae from individual primary recipients were transplanted into secondary irradiated recipients. Because the cell content of a tibia is approximately 0.6 that of a femur,20 such suspensions were assumed to contain the progenitor content of 3.2 femurs. The cell equivalent of one-fifth of a femur was injected into 5 or 6 recipients and donor-derived hematopoietic reconstitution was then determined 10 and 20 weeks later.

Statistical analysis. The Student's t-test analysis was used for the comparison of primary recipients of FL cells with their adult BM counterparts, with respect both to the mean percentage of donor-derived nucleated peripheral blood cells (at each of the 3 time points of analysis) and the mean BM cellularity, as well as for the comparison of secondary recipients of BM from primary adult BM recipients versus that of primary FL recipients, with regards to the mean percentage of donor-derived repopulation. A Pearson-χ^2 test was used to analyze whether the transplantation of BM from primary adult BM recipients compared with that from primary FL recipients resulted in a significantly different outcome, defined as no positive mice versus any positive mice, in the groups of secondary recipients. To analyze whether there was a relationship between the proportion of Ly-5.1+ cells in the primary recipient and that of the secondary recipient, a linear regression analysis was used. There was no significant difference in the slope of the regression lines of this relationship when serially transplanted Ly-5.1+ FL cells were compared with such adult BM cells. Therefore, a regression line with the same slope (found to be not significantly different from 0; β = 0.16, P = .35) but a different intercept was fit on each set of data.

RESULTS

Donor-derived reconstitution of primary recipients transplanted with FL or adult BM cells. Mice injected with 0.5 to 5 CRU, each of either FL or adult BM origin, were analyzed for donor-derived hematopoietic reconstitution 4, 8, and 16 weeks later. The results are presented in Tables 1 and 2, respectively, and in Fig 1 the changes in donor-derived nucleated peripheral blood cells that occurred over time are plotted to show the different kinetics of repopulation in groups of recipients of similar numbers of FL and adult BM CRU. Despite the large standard deviations observed at every time point reflecting the extensive variability between individual recipients in each group as expected from previous studies of clonal repopulation by both sources of stem cells,21-24 the average extent of donor-repopulation in the recipients of FL CRU was significantly (P < .02) higher in all 4 groups compared with adult BM recipients, both at 8 and 16 weeks after transplantation.

To reduce the contribution of intrinsic clonal variation to the analysis of potential differences between FL and adult BM CRU, a comparison was made of the number of animals in which there was an increase in donor-derived repopulation over time when each was tracked individually. The results of this analysis are shown in Table 3. The most dramatic difference was then found to occur between 4 and 8 weeks after the transplantation, with both the number of animals that displayed an increase in percentage donor-repopulation (~80%) and the extent of the increase (up to 63%) being greater in recipients of FL CRU. In contrast, in recipients that had a small chance of receiving greater than 1 adult BM CRU (groups 1A, P = .04; groups 2A, P = .18), only 38% and 15%, respectively, showed an increase, which did not exceed 23% in any given recipient. Even in groups 3A and 4A, in which the chance of having received greater than 1 CRU was 0.46 and 0.85, the percentage of animals that showed an increase in donor-derived reconstitution was only 29% and 57%, respectively. Overall, only ~35% of recipients of adult BM cells showed an increase in donor-derived reconstitution after 4 weeks, as compared with ~80% of recipients of FL cells.

Cell counts of BM samples from primary recipients also showed that there was a significantly (up to 2-fold) higher BM cellularity in the recipients of a FL CRU than in the recipients of an adult BM CRU (results not shown). From these observations it was concluded that the transplantation...
Table 1. Contribution of FL CRU to Total Mature Blood Cell Pool as a Function of CRU Input and Time Posttransplantation

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. and Type of Cells Transplanted</th>
<th>Chance of Having</th>
<th>% Donor-Derived Peripheral Blood Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of CRU/Mouse</td>
<td>Receiving &gt;1</td>
</tr>
</tbody>
</table>
| 1         | 8,000 total FL, 10, 15 or 20 Sca-
|           | -1-Lin-1, 10 Sca-1+/L. /H2-K- , and 200
|           | Sca-1+/Lin-1/WGA- cells           |22              | 0.4 ± 0.02 | 0.058 ± 0.007 | 11 ± 3 | 22 ± 4 | 34 ± 5 |
| 2         | 16,000 or 10,000 total FL, 500 total Sca-1-, 50, 30, or 35 (low SSC) Sca-
|           | -1-, 30 Sca-1-/Lin-1-/H2-K- , and 20 Sca-1-/Lin-1-/H2-
|           | K- cells                          |36              | 0.7 ± 0.02 | 0.16 ± 0.008  | 18 ± 2 | 32 ± 3 | 33 ± 4 |
| 3         | 25,000 or 20,000 total FL, 70 (low SSC) Sca-1-,
|           | 40 Sca-1-/Lin-1-, 40 Sca-1+/Lin-
|           | -/CD71-, or 40 Sca-1+/Lin-1-/H2-
|           | K- cells                          |17              | 1.3 ± 0.05 | 0.38 ± 0.02  | 26 ± 5 | 56 ± 6 | 64 ± 6 |
| 4         | 40,000 total FL, 2,000 total Sca-1,
|           | or 150 (low SSC) Sca-1- cells     |17              | 2.6 ± 0.07 | 0.73 ± 0.02  | 44 ± 4 | 61 ± 6 | 60 ± 7 |
| 5         | 100,000 total FL or 300 (low SSC) Sca-1- cells. |20              | 5.8 ± 0.01 | 0.98 ± 0.00  | 42 ± 3 | 57 ± 5 | 62 ± 5 |

Values shown are the mean ± SEM.

of FL CRU not only results in a higher percentage of donor-
derived reconstitution on a per CRU basis but also results
in a higher absolute number of nucleated hematopoietic cells
in the BM of such recipients.

Donor-reconstitution of secondary recipients of BM from
primary recipients of adult BM or FL transplants. To in-
vestigate whether transplanted FL CRU, after establishing a
steady-state hematopoiesis in primary recipients (>6 months
after the transplantation25), would continue to show a higher
proliferative potential upon transplantation of their progeny
in secondary recipients, primary recipients from each group
were selected at random and their BM cells analyzed as
described in the Materials and Methods. The extent of repop-
ulation of the secondary recipients by cells from the original
FL or BM CRU transplanted into the primary recipients that
was identified by anti-Ly-5.1 staining 20 weeks after the second-
ary transplants were performed is shown in Table 4. A few
of the secondary recipients with greater than 1%
Ly-5.1+ peripheral blood cells were not scored as positive
because the donor-reconstitution was restricted to the
lymphoid compartment. Most likely these cells represent
long-lived B and T cells,17,25 a population of cells that be-
comes more noticeable when large numbers of donor-cells
are injected. The outcome, ie, groups with no positive mice
or groups with any positive mice, after the transplantation of
BM from primary FL recipients was found to be significantly
different from that after the transplantation of BM from adult
BM primary recipients (P < .01).

All except 2 of the groups of mice that received BM
from primary recipients of FL showed detectable levels of
multilineage reconstitution by cells of FL origin, whereas
only 4 of 12 groups of mice injected with BM cells from
primary recipients of adult BM CRU showed further transfer
of CRU activity. Of the 4 positive groups of secondary recip-
ients that were transplanted with BM from a primary recipi-
ent of adult BM cells, only 2 showed comparable levels of
Ly-5.1+ nucleated peripheral blood cells to the secondary
recipients of previously transplanted FL cells. Statistical
analysis showed that there was a significant difference in
the level of Ly-5.1+ reconstitution in secondary recipients
between the groups of serially transplanted Ly-5.1+ FL cells
and such adult BM cells (P < .008). However, a relationship
between the proportion Ly-5.1+ cells in the primary recipient
and that of the secondary recipient could not be shown in
either group. Interestingly, compared with the analysis per-
formed at week 10, 71% of the repopulated secondary recipi-
ents of Ly-5.1+ FL cells and such adult BM cells (P < .008). However, a relationship
between the proportion Ly-5.1+ cells in the primary recipient
and that of the secondary recipient could not be shown in
either group. Interestingly, compared with the analysis per-
formed at week 10, 71% of the repopulated secondary recipi-
ents of Ly-5.1+ FL cells showed a decline in Ly-5.1+ hemat-
opoietic reconstitution at week 20, whereas only 37% of
the adult BM secondary recipients did so (data not shown).

DISCUSSION

The results of many previous studies suggest that hematopoietic stem cell recruitment in vivo depends on the interplay
of a number of variable parameters, some of which are intrin-
sic to the stem cell itself, as well as others that represent
changes in the microenvironment to which the stem cell is
FETAL VERSUS ADULT STEM CELL TRANSPLANTATION

Table 2. Contribution of Adult BM CRU to Total Mature Blood Cell Pool as a Function of CRU Input and Time Posttransplantation

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. and Type of Cells Transplanted</th>
<th>No. of CRU/ Mouse</th>
<th>Chance of Having Received &gt;1 CRU</th>
<th>% Donor-Derived Peripheral Blood Cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>8,000, 6,500, or 4,000 total BM, 5 Sca-1'/Lin-'/WGA**+, 50 Sca-1'/Lin-'/WGA**+/c-Kit, or 90 or 30 Sca-1'/Lin-'/WGA**+/Rh123**+ cells.</td>
<td>12</td>
<td>0.3 ± 0.06</td>
<td>0.04 ± 0.009</td>
</tr>
<tr>
<td>2A</td>
<td>15,000 or 12,000 total BM, 30 or 20 Sca-1'/Lin-'/WGA**+, 10 Sca-1'/Lin-'/WGA**+/c-Kit+, or 40 Sca-1'/Lin-'/WGA**+/M2- K** cells.</td>
<td>38</td>
<td>0.8 ± 0.02</td>
<td>0.18 ± 0.007</td>
</tr>
<tr>
<td>3A</td>
<td>30,000 total BM, 60 or 40 Sca-1'/Lin-'/WGA**+, 15 Sca-1'/Lin-'/WGA**+/Rh123**+, or 30 Sca-1'/Lin-'/WGA**+/c-Kit+ cells.</td>
<td>23</td>
<td>1.6 ± 0.06</td>
<td>0.46 ± 0.02</td>
</tr>
<tr>
<td>4A</td>
<td>60,000 total BM, 100 Sca-1'/Lin-'/WGA**+, or 45 Sca-1'/Lin-'/WGA**+/Rh123**+ cells.</td>
<td>18</td>
<td>3.4 ± 0.07</td>
<td>0.84 ± 0.01</td>
</tr>
</tbody>
</table>

Values shown are the mean ± SEM.

* At certain time points of analysis the difference in the percentage of donor-derived nucleated peripheral blood cells in groups of recipients of adult BM transplants was compared with their FL counterpart, significantly lower.

† P < .02.

‡ P < .01.

§ P < .002.

|| P < .001.

exposed. Because suitable assays for measuring the behavior of individual stem cells with long-term in vivo repopulating potential were not available until relatively recently,25,26 not much is known about the nature of any of these parameters. In the present study, we measured the number of mature blood cells produced by low numbers of FL CRU (<5) following their transplantation into heavily irradiated recipients as a strategy to compare the proliferative behavior of CRU from FL as compared with adult BM origin. The results clearly show that, between 4 and 8 weeks posttransplant, FL CRU produce larger clones of mature progeny than their BM counterparts. Additional support for a higher proliferative potential of fetal liver CRU is provided by the finding of a significantly higher BM cellularity in the recipients of FL as compared with adult BM CRU. Moreover, transplantation into secondary recipients of the cells generated from the CRU transplanted into primary recipients showed that more CRU were present in primary recipients of FL cells. These results are not explained by an increased number of stem cells in fetal liver grafts contributing to late but not early engraftment, because the fraction of animals that did not show any sign of donor cell engraftment at 4 weeks and 4 months after transplantation were identical.

There are in vitro and in vivo reports describing a greater proliferative activity of FL cells in comparison to adult BM cells.15,27,32; however, there are only three that assayed long-term repopulating activity of FL cells by comparison to adult BM; interestingly, they gave conflicting results.28,30 Micklem et al.30 found that, when equal numbers of FL and adult BM cells were transplanted in the same irradiated recipient, the FL cells would outcompete the adult BM cells. The main difference in hematopoietic reconstitution occurred in the first 8 to 10 weeks posttransplantation, after which a more stable balance between FL- and adult BM-derived cells was seen, although analysis of a few recipients at much later time points (up to 15 months posttransplantation) indicated that the contribution of FL-derived cells continued to increase. On the other hand, Harrison et al.28,29 found no difference in the long-term repopulating ability of BM and FL cells based on an assessment of red blood cell reconstitution. A possible explanation for these results may lie in the fact that a higher number of cells were transplanted in the latter experiments. In the present studies, we noted that the differences in the level of donor-derived repopulation by FL CRU and adult BM CRU decreased when the numbers of CRU transplanted into the recipient was increased (ie, compare in Tables 1 and 2, groups 1 and 4 with 1a and 4a, respectively).

Serial transplantation of adult BM cells have shown that the repopulating ability of the stem cells present in the original input population is reduced to undetectable levels within 4 to 6 passages33,34 and even faster when the number of stem cells initially transplanted is low.36-38 In the present study, when transplants containing less than 3 CRU from adult BM were subjected to 2 rounds of transplantation, only occasional cells with long-term reconstituting ability could be detected in the cells transferred from the primary to the
secondary recipients, whereas the presence of such cells was a consistent feature of primary recipients of equivalent numbers of FL CRU. This feature may in part be a consequence of the difference in the number of BM cells transplanted. By using a standard dose of one-fifth of a femur, secondary recipients of BM from primary recipients of adult BM cells received fourfold to fivefold less cells than secondary recipients of similarly treated FL cells (resulting from a BM cellularity that was on average 1.8-fold lower combined with a 2- to 3-fold lower percentage of Ly-5.1+ cells). However, if cellularity alone was responsible for the observed differences, one would still expect to see a few "positive" animals in groups of secondary recipients of marrow from previously transplanted adult BM CRU. Additional evidence that a dif-

| Table 3. Changes in Percentage of Donor-Derived Nucleated Peripheral Blood Cells in Recipients of FL Transplants Compared With Recipients of Adult BM Transplants |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Group* | n | % | Increase (range) | Average ± SD | Decrease (range) | Average ± SD | Group* | n | % | Increase (range) | Average ± SD | Decrease (range) | Average ± SD |
| 1 | 21 | 76 | 15 ± 13 (1-38) | 14 | 5 ± 4 (2-7) | 22 | 18 ± 17 (2-48) | 9 | 6 ± 4 (2-11) |
| 1A | 8 | 38 | 5 ± 3 (2-6) | 62 | 2 ± 1 (2-4) | 12 | 42 ± 10 (3-16) | 16 | 3 ± 2 (1-6) |
| 2 | 33 | 82 | 19 ± 16 (1-50) | 9 | 5 ± 6 (2-12) | 36 | 44 ± 15 (2-47) | 50 | 11 ± 8 (1-27) |
| 2A | 13 | 15 | 19 ± 6 (14-23) | 54 | 8 ± 7 (2-24) | 33 | 18 ± 14 (2-26) | 50 | 5 ± 5 (1-20) |
| 3 | 17 | 82 | 37 ± 17 (6-63) | 12 | 6 ± 2 (5-7) | 17 | 65 ± 15 (10-4-36) | 17 | 10 ± 7 (6-18) |
| 3A | 7 | 29 | 13 ± 10 (6-20) | 57 | 5 ± 3 (1-8) | 23 | 43 ± 11 (3-29) | 44 | 5 ± 5 (1-18) |
| 4 | 17 | 82 | 24 ± 10 (4-40) | 12 | 22 ± 12 (14-31) | 17 | 53 ± 8 (5-1-18) | 41 | 14 ± 11 (2-29) |
| 4A | 7 | 57 | 9 ± 5 (5-15) | 43 | 9 ± 5 (3-13) | 18 | 45 ± 9 (6-2-16) | 33 | 6 ± 5 (1-15) |

* The identification of each group is according to that outlined in Tables 1 and 2.
Table 4. Percentage of Donor-Derived Nucleated Peripheral Blood Cells in Secondary Recipients of Marrow (1/5 Femur) From Primary Recipients of Limiting Numbers of FL Cells or Adult BM Cells

<table>
<thead>
<tr>
<th>CRU Received</th>
<th>1st Recipient</th>
<th>2nd Recipient</th>
<th>Pos. Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Repop. t</td>
<td>% Repop. t</td>
<td></td>
</tr>
<tr>
<td>2.9</td>
<td>88</td>
<td>67</td>
<td>76 84 89</td>
</tr>
<tr>
<td>2.9</td>
<td>81</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2.9</td>
<td>69</td>
<td>14</td>
<td>20 21 14 15 5/56</td>
</tr>
<tr>
<td>1.4</td>
<td>78</td>
<td>23</td>
<td>51 44 28 23 5/56</td>
</tr>
<tr>
<td>1.0</td>
<td>88</td>
<td>40</td>
<td>20 33 20 23 5/56</td>
</tr>
<tr>
<td>0.7</td>
<td>43</td>
<td>24</td>
<td>22 20 28 40 35 6/65</td>
</tr>
<tr>
<td>0.7</td>
<td>84</td>
<td>88</td>
<td>73 69 67 73 82 6/65</td>
</tr>
<tr>
<td>0.6</td>
<td>60</td>
<td>48</td>
<td>18 47 41 52 5/56</td>
</tr>
<tr>
<td>0.6</td>
<td>29</td>
<td>50</td>
<td>31 58 33 64 5/56</td>
</tr>
<tr>
<td>0.6</td>
<td>19</td>
<td>4</td>
<td>1 1 1 0 0/5</td>
</tr>
<tr>
<td>0.6</td>
<td>26</td>
<td>2</td>
<td>1 1 — 0/3</td>
</tr>
<tr>
<td>0.6</td>
<td>10</td>
<td>33</td>
<td>26 61 29 — 4/44</td>
</tr>
<tr>
<td>0.1</td>
<td>57</td>
<td>34</td>
<td>35 29 29 23 43 6/65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CRU Received</th>
<th>1st Recipient</th>
<th>2nd Recipient</th>
<th>Pos. Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Repop. t</td>
<td>% Repop. t</td>
<td></td>
</tr>
<tr>
<td>2.9</td>
<td>22</td>
<td>0</td>
<td>0 0 1 0 0/5</td>
</tr>
<tr>
<td>2.9</td>
<td>10</td>
<td>0</td>
<td>3 1 1 0 0/5</td>
</tr>
<tr>
<td>2.9</td>
<td>6</td>
<td>0</td>
<td>1 1 0 0 0/5</td>
</tr>
<tr>
<td>1.1</td>
<td>17</td>
<td>10</td>
<td>13 3 5 4 — 3/45</td>
</tr>
<tr>
<td>1.1</td>
<td>59</td>
<td>0</td>
<td>0 0 0 0 0/5</td>
</tr>
<tr>
<td>1.1</td>
<td>23</td>
<td>56</td>
<td>34 52 47 15 15 5/56</td>
</tr>
<tr>
<td>0.9</td>
<td>81</td>
<td>0</td>
<td>1 1 0 0 0/5</td>
</tr>
<tr>
<td>0.9</td>
<td>15</td>
<td>0</td>
<td>0 0 0 0 0/5</td>
</tr>
<tr>
<td>0.9</td>
<td>64</td>
<td>3</td>
<td>2 3 2 4 4/45</td>
</tr>
<tr>
<td>0.7</td>
<td>3</td>
<td>0</td>
<td>0 0 0 0 — 0/4</td>
</tr>
<tr>
<td>0.6</td>
<td>93</td>
<td>—</td>
<td>— — — — — —</td>
</tr>
<tr>
<td>0.2</td>
<td>15</td>
<td>0</td>
<td>2 1 1 1 0 0/5</td>
</tr>
</tbody>
</table>

* The primary recipients were taken from a group of animals that had received on average the indicated number of CRU, which was calculated as described in the Materials and Methods.
† The percentage of donor-derived (Ly-5.1+) nucleated peripheral blood cells (% repop.) was determined from every primary recipient before killing for donating BM. The presented percentages of Ly-5.1+ nucleated peripheral blood cells from the secondary recipients were determined 20 weeks after the transplantation. Secondary recipients that did not survive (death occurred between 14 and 24 days after the transplantation) are indicated by a dash.
‡ An animal was designated as “positive” (pos.) when the Ly-5.1- cells represented greater than 1% of all the nucleated peripheral blood cells and were detectable in both myeloid and lymphoid population as determined by their side scatter properties. Groups with positive animals are indicated by §.

The number of transplanted cells is not the only explanation for the observations provided by the finding that only 1 of 4 recipients showed multilineage repopulation (8.6% Ly-5.1+ cells) upon transplantation with fivefold the usual cell number (ie, equal of 1 femur) of BM from primary recipients that were previously transplanted with adult BM CRU. In both the FL and adult BM primary recipients, the extent of donor-derived repopulation was not predictable for the hematopoietic contribution by the original input population in the secondary recipients (Table 4). This is comparable to previous observations that showed no correlation between self-renewal capacity and the size of the primary colony (CFU-C and CFU-S40 A').

The results of this study clearly show an intrinsic difference between FL and adult BM cells that meet the definition of CRU and suggest potential heterogeneity within this compartment that may require more stringent criteria to show. An attractive explanation for the differences observed would be that FL CRU have a higher intrinsically determined probability to undergo self-renewal divisions upon being stimulated to proliferate in an adult BM microenvironment. However, potential differences in cell cycle times or the number of stem cells recruited or maintained could also contribute to the differences seen. In this regard, it is interesting to note that Sca-1-Lin-~hThy-1.1+ cells from FL have twice as many cells actively cycling than adult BM cells with this phenotype and colony-forming cells from day 16 to 17 FL have been shown to have a significantly shorter doubling time than similar cells from adult BM. These results suggest that FL and adult BM HSCs do not only differ in the number of divisions they can undergo, based on their replicative history, but that there may be other factors that influence their proliferative behavior. Unraveling the underlying mechanisms of the differences displayed by adult BM and FL HSCs in long-term reconstituting properties may elucidate important regulatory mechanisms involved in self-renewal versus differentiation. Irrespective of the nature of these regulatory mechanisms, this study shows the possible therapeutic advantages, in addition to the immaturity of immune-reactive cells, of using FL cells as a source of hematopoietic cells. FL cells may be especially attractive for stem cell-based gene therapy strategies, because it has also been shown that the stem cell-containing population in the FL contains twice as many cells in the S/G2/M phase as the phenotypically similar population in adult BM. To exploit the potential of fetal stem cells for transplantation it will be important to establish procedures that will enhance their engraftment.

ACKNOWLEDGMENT

We thank Gayle Thornbury and Wieslawa Dragowska for assistance with the flow cytometry, Dr John Spinelli for statistical advice, and Jessica Maltman and Maya Sinclair for assistance with the animal procedures.

REFERENCES


4. Palacios R, Imhof BA: At day 8-8.5 of mouse development the yolk sac, not the embryo proper, has lymphoid precursor potential in vivo and in vitro. Proc Natl Acad Sci USA 90:6581, 1993


41. Gregory CJ, Henkelman RM: Relationship between early he-
mopoietic progenitor cells determined by correlation analysis of their numbers in individual spleen colonies, in Baum SJ, Ledney GD (eds): Experimental Hematology Today. New York, NY, Springer-Verlag, 1977, p 93
The repopulation potential of fetal liver hematopoietic stem cells in mice exceeds that of their liver adult bone marrow counterparts

VI Rebel, CL Miller, CJ Eaves and PM Lansdorp