The quality of long-term hematopoietic engraftment after bone marrow transplantation (BMT) has not been well characterized. Clinical autologous BMT involves removal of <5% of the total content of the recipient marrow followed by ablation of the remaining marrow and reinfusion. To study long-term consequences of transplanting limited numbers of BM stem cells further, we evaluated the hematopoietic reserve in recipient animals after transplantation of varying quantities of BM. Recipient animals demonstrated a donor BM cell dose-dependent decrease in stem cell content and self-renewal capacity that was not reflected in peripheral blood (PB) counts or BM cellularity. This decrease was observed after initial BM recovery and did not change with time after transplantation, demonstrating a permanent loss in BM self-renewal capacity. In addition, animals alive at 3 months, a time selected to allow BM recovery, also demonstrated a donor BM cell dose-dependent decrease in survival at 1 year. These results emphasize the importance of optimizing stem cell number in BMT.

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MARROW STEM CELL SELF-RENEWAL FOLLOWING BMT

Table 1. Cells per Hind Limb x 10^6 After BMT

<table>
<thead>
<tr>
<th>Time After BMT (mo)</th>
<th>Age-Matched Nontransplant Controls (10^7)</th>
<th>1 x 10^6</th>
<th>1 x 10^8</th>
<th>1 x 10^9</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>23 ± 0.7</td>
<td>22 ± 5.2</td>
<td>21 ± 2</td>
<td>23 ± 2.9</td>
</tr>
<tr>
<td>6</td>
<td>33 ± 10</td>
<td>25 ± 7.8</td>
<td>31 ± 8.2</td>
<td>28 ± 8.5</td>
</tr>
<tr>
<td>9</td>
<td>24 ± 1.2</td>
<td>26.7 ± 2.1</td>
<td>23.7 ± 3.4</td>
<td>23.7 ± 1.4</td>
</tr>
<tr>
<td>Total</td>
<td>26.6 ± 3.2</td>
<td>24.7 ± 2.7</td>
<td>25.0 ± 2.9</td>
<td>24.9 ± 2.6</td>
</tr>
</tbody>
</table>

Values are the mean ± SE of three separate experiments for each time point (n = 3). Each experimental point pooled marrow from three to four mice. Each total represents data from nine experiments (n = 9). The degree of statistical significance is listed for the total means for the 10^7, 10^8, or 10^9 groups as compared with the age-matched control group (control v.10^7, 10^8, or 10^9) and within each of the transplanted groups (10^7, v.10^8, 10^9, or v.10^9).

RESULTS

BM cellularity per hind limb at 3, 6, and 9 months after BMT is shown in Table 1. Transplanted animals demonstrated BM cellularity similar to that noted in age-matched nontransplanted control animals. No BM cellularity differences were noted as a function of the number of cells transplanted.

The number of CFUs per hind limb at 3, 6, and 9 months after BMT is shown in Table 2. Transplanted animals receiving 1 x 10^6 or 1 x 10^8 cells had a significantly lower BM CFUs content per hind limb as compared with age-matched, nontransplanted control animals. Animals receiving 1 x 10^8 cells also had a significantly decreased CFUs content as compared with animals receiving 1 x 10^8 or 1 x 10^9 cells. There was no significant difference between animals receiving 1 x 10^8 cells and age-matched, nontransplanted control animals. The decrease in CFUs content was noted at 3, 6, and 9 months after BMT and did not recover with time.

BM CFUs self-renewal at 3, 6, and 9 months after BMT is shown in Table 3. Transplanted animals had a significantly lower marrow CFUs self-renewal capacity (Rs) as compared with nontransplanted control animals. This decrease was most pronounced in the 10^8 BM group but was also noted in animals after BMT with 10^6 or 10^9 cells. Significant differences were noted between all groups. These differences persisted to 9 months after BMT and demonstrated no evidence for recovery with time.

Survival of animals starting at 3 months after BMT was donor BM cell dose dependent; animals transplanted with 10^6 cells had the lowest survival rate (Fig 1). Survival curves were significantly different from each other and from age-matched control curves. Although these results are consistent with the hypothesis of limited BM reserve, we do not know whether the cause of death has anything to do with exhaustion of the proliferative potential of transplanted hematopoietic stem cells (thrombocytopenia, anemia, infection), other transplanted progenitors (stroma), or mechanisms currently obscure. What is clear is that long-term survival is directly related to the number of BM cells injected.

DISCUSSION

The BM stem cell compartment appears to have an organizational structure.6,10,13,18,24 This organization provides control for replication and differentiation of stem cells and allows protection of the earliest stem cells throughout the animal’s life.

There are several models for selection of stem cells for commitment to differentiation. The clonal succession model proposes that individual stem cells are selected for clonal expansion, with resulting production of differentiated cells, and that only a few stem cells are clonally active at a specific time.6,25,26 This model has been supported by both in vitro and in vivo studies that used cell markers to follow specific clonal activity. In contrast, another model studying BM after transplantation supports the mechanism of continued activity of all pluripotent stem cells during the recipient’s life span.29

A model for organization of the stem cell compartment

Table 2. CFU Content per Hind Limb

<table>
<thead>
<tr>
<th>Time After BMT (mo)</th>
<th>Cells Transplanted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^7</td>
</tr>
<tr>
<td>3</td>
<td>2.973 ± 360</td>
</tr>
<tr>
<td>6</td>
<td>2.838 ± 629</td>
</tr>
<tr>
<td>9</td>
<td>3.159 ± 80</td>
</tr>
<tr>
<td>Total</td>
<td>2.990 ± 204</td>
</tr>
</tbody>
</table>

*Only two experiments were performed. Values represent the mean ± SE of three separate experiments for each time point (n = 3). Each experimental point pooled marrow from three to four mice. Each total represents data from eight to nine experiments (n = 8 or 9). The degree of statistical significance is listed for the total means for the 10^7, 10^8, or 10^9 groups as compared with the age-matched control group (control v.10^7, 10^8, or 10^9) and within each of the transplanted groups (10^7, v.10^8, 10^9, or v.10^9).
which accounts for in vitro and in vivo findings suggests different mechanisms used to meet different circumstances.\textsuperscript{18} In circumstances in which a few stem cells are required to repopulate the compartment, all are subject to proliferative stress and cell division, thus decreasing the average self-renewal capacity. This process is observed in vitro during the first 3 weeks of culture,\textsuperscript{17} in utero,\textsuperscript{3} and after BMT.\textsuperscript{28-29} Our current study data suggest that the smaller the number of stem cells transplanted the greater the decrease in average self-renewal observed. In contrast, with an intact BM compartment, end-cell proliferation requires only limited primitive CFUs activity, and clonal succession obtains. Stem cells are recruited as needed without continued proliferative activity of all stem cells with loss of self-renewal, BM once the stem cell pool reexpands.\textsuperscript{28} Thus, after initial cultures,\textsuperscript{28} during the life span of the mouse,\textsuperscript{3} and after BMT,\textsuperscript{29} stem cell self-renewal capacity has been measured by either excision of individual spleen colonies and determination of daughter CFUs,\textsuperscript{2} by serial transplantability,\textsuperscript{6} or by Rs,\textsuperscript{6} a measurement of CFUs proliferation in 14 days. Spleen colonies have been commonly measured at eight to 12 days after lethal irradiation and tail-vein injection of a measured number of marrow cells. Day-11 to day-12 colonies appear to be more primitive than day-8 colonies, and the ability of individual colonies to produce daughter colonies (self-renewal) increases with colony size and age.\textsuperscript{31-33} None-

\begin{table}
\centering
\caption{CFU Self-Renewal Capacity (Rs)}
\begin{tabular}{|c|c|c|c|c|}
\hline
Time After BMT (mo) & Age-Matched Nontransplanted Cells Transplanted & \multicolumn{3}{c|}{Age-Matched Nontransplanted Cells Transplanted} \\
& (10^7) & 10^4 & 10^5 & 10^6 \\
\hline
3 & 37 ± 7.8 & 15 ± 11 & 7.4 ± 3.4 & 2.2 ± 0.8 \\
6 & 29.8 ± 4.9 & 13.0 ± 3.9 & 6.6 & 1.6 ± 0.8 \\
9 & 26.7 ± 8.5 & 21.2 ± 12.1 & 7.9 ± 2.9 & 1.3 ± 0.5 \\
\hline
Total & 34.6 ± 4.6 & 16.4 ± 5.7 & 7.4 ± 1.8 & 1.7 ± 0.4 \\
\hline
\end{tabular}
\textsuperscript{*}Only two experiments were performed. Values represent the mean ± SE of three separate experiments for each time point (n = 3). Each experimental pool marrow from three to four mice. Each total mean represents data from eight to nine experiments (n = 8 or 9). The degree of statistical significance is listed for the overall means for the 10^7, 10^6, and 10^5 groups as compared with the age-matched control group (control v 10^7, 10^6, or 10^5) and within each of the transplanted groups (10^7 v 10^6, 10^5 v 10^6, or 10^5 v 10^6). Statistically significant differences between total means were present for all the above comparisons. No statistical difference was seen at 9 months between the age-matched nontransplant control and the 10^7 transplanted group.

\end{table}

Fig 1. Survival of transplanted animals receiving 10^7, 10^6, or 10^5 cells as compared with nontransplanted age-matched control animals. Only animals surviving the transplantation procedure at 3 months after BMT were considered for survival analysis. Statistical significance was as follows: 10^7 v control, P < .001; 10^6 v control, P < .001; 10^5 v 10^7, P < .001; 10^5 v 10^6, P = .01.

None-theless, the day-11 to day-12 colony assay does not directly measure self-renewal and, despite the more primitive nature of day-11 to day-12 colonies, the ratio of the number of day-11 to day-12 to day-8 colonies is a poor reflection of BM self-renewal as compared with the excision colony method\textsuperscript{34} or with serial transplantability or Rs techniques (P. Mauch, unpublished observations, 1988). In the current experiments, eight-day CFUs were measured. Perhaps even more profound decrease in stem cell content would have been noted in the 10^5 and 10^6 transplanted groups if day-11 CFUs had been measured. However, the stem cell self-renewal assay (Rs) provided a good correlation between low numbers of stem cells transplanted and decrease in survival and was able to measure directly the decrease (loss) in self-renewal capacity, a measurement not available from 11-day CFUs assay.

Recipient stem cell recovery in animals receiving 1 x 10^5 cells was not observed in this study using a C57BL/6 congeneric hemoglobin marker. Previous research by our group has demonstrated that host BM recovery in recipients of congeneric BM can be abrogated by increasing the cell dose transplanted.\textsuperscript{35} Thus, with the absence of BM recovery with
Marrow stem cell self-renewal following BMT

1 x 10^6 cells transplanted, we would not expect to see such recovery with 1 x 10^4 or 1 x 10^5 cells.

These results have important clinical implications for BMT. Despite seemingly adequate numbers of BM cells and formed blood elements in transplanted recipients, transplantation with smaller numbers of stem cells results in a decrease in BM stem cell content and, perhaps more important, a reduced proliferative capacity of those stem cells. This reduced capacity may influence host tolerance to cytotoxic agents or other circumstances which require subsequent proliferative demand and may be associated with a decreased long-term survival. Efforts must be made to transplant the largest number of stem cells possible. When feasible, they should be harvested before any large proliferative requirement is made of them.

REFERENCES

Loss of hematopoietic stem cell self-renewal after bone marrow transplantation

P Mauch and S Hellman