BRIEF REPORT

Initial Investigations of the Changes in the Stem Cell Compartment of Murine Bone Marrow During Post-Hypoxic Polycythemia

By J. M. Hurst, M. S. Turner, J. M. Yoffey and L. G. Lajtha

THE INVERSE RELATIONSHIP between the number of bone marrow lymphocytes and degree of erythropoiesis seen in guinea pigs during and after exposure to simulated high altitude suggested that marrow lymphocytes might play a functional role in erythropoiesis.1 These findings were later duplicated in mice exposed to the same conditions.2 The hypothesis that lymphocytes are involved in erythropoiesis was substantiated by Cudkowicz, who showed that the ability of marrow grafts to restore hemopoiesis in irradiated recipients was directly proportional to the lymphocyte content of the graft.3

Quantitative bone marrow studies of guinea pigs1 and mice2 during the erythropoietic depression following hypoxia ("rebound") showed that accumulation of bone marrow lymphocytes followed a specific, reproducible pattern reaching twice normal at seven days after removal from the hypoxic environment. It is well known that if erythropoietic activity is suppressed (by transfusion or by temporary exposure to hypoxia), the response to a given dose of erythropoietin is greater than the normal response. If erythropoietin causes differentiation of stem cells into the erythroid line, as is generally agreed, it may be assumed that stem cells accumulate during suppression of erythropoiesis.

There has been some disagreement4,5 about the proposed existence of an "erythropoietin-sensitive" stem cell as distinct from the "CFU" of McCulloch and Till.6 In this experiment, the colony-forming property of "stem cells" was assayed in bone marrow obtained from mice during various phases of post-hypoxic polycythemia, in order to find whether a correlation could be established between the accumulation of lymphocytes accompanying erythropoietic depression and the relative size of the stem cell pool.
MATERIALS AND METHODS

Polycythemic Donor Animals

Male F₁ (AKR × C3H) mice 12–14 weeks old were used in all experiments. Animals were prepared for the assay by being kept in a decompression chamber at a simulated altitude of 17,000 feet continuously for five days, after which they were returned to ambient air. Femoral marrow obtained from animals at various times after removal from the chamber and from normal controls was assayed for its stem cell content (see assay procedure below). Nucleated cell content of the marrow, reticulocytes, and 48 hour ⁵⁹Fe incorporation into peripheral blood were obtained in a parallel experiment the results of which were published in graph form,² but are summarized here in numerical form (Table 1) to indicate the erythropoietic status of the donor animals at the time their marrows were being assayed for stem cells. Myelograms were performed on marrow from the left femur of each donor while the right femur was being prepared for grafting. The ratios of marrow cells were used to calculate the actual number of various cell types in the marrow graft. The same ratios, together with the total marrow cellularity values obtained in the earlier experiment, were used to calculate the cellular content per milligram of donor marrow. The total number of lymphocytes (small lymphocytes and transitional cells) obtained from myelograms in this experiment was similar to the number obtained in the parallel experiment.

Stem Cell Assay

Assays were performed on marrow from mice at 1, 3, 5, 7, and 9 days after removal from the decompression chamber (rebound) and in normal controls. Each of the six experimental groups consisted of 4 donors and 10 irradiated recipients. Marrow from each set of donors was pooled and suspended in tissue culture media 199 (Grand Island Biological Co.). After thoroughly agitating the suspension, clumps and debris were allowed to settle, the supernatant cells withdrawn and diluted (after hemocytometric count) to 5 × 10⁶ nucleated cells/ml. Two tenths ml. of this suspension (10⁵ nucleated cells) was injected into the tail vein of each recipient 2–3 hours after irradiation. Irradiation was accomplished with a ⁰⁶cobalt source and was delivered at a dose rate of 74.6 rads per minute. Each side of a circular, partitioned Perspex chamber containing 10 immobilized mice (unanesthetized) was exposed to 450 rads, for a total dose of 900 rads. The dose rate at the perimeter of the chamber was approximately 5 per cent less than the calculated dose to the middle center of the chamber. Spleens were removed from test animals 9 days after irradiation and graft, weighed, and fixed in Bouin’s solution according to the method of McCulloch and Till.⁶ Macroscopically visible colonies on the top surface of the spleens were counted by two workers and the average reading recorded.

RESULTS

Erythropoietic activity of the donor animals decreased progressively during rebound as indicated by the per cent radio-iron incorporation into circulating blood and the nucleated erythrocyte and reticulocyte content of their bone marrows (Table 1). Table 2 shows that during this period the marrow lymphocytes (small lymphocytes and transitional cells) gradually increased from less than normal at one day rebound to twice normal at 7 days rebound. The stem cell content of rebound marrows as reflected by their ability to form spleen colonies in irradiated recipients progressively increased to more than twice the normal value by 7 days of rebound and was generally proportional to the increase in lymphocyte content (Fig. 1). Correspondingly, the colony-forming capacity of rebound marrow was generally related to the degree of depression in its erythropoietic activity.
Table 1.—Erythropoietic Status of Donor Mice

<table>
<thead>
<tr>
<th>Day Rebound</th>
<th>No. Animals</th>
<th>48 Hour per cent 59Fe Incorporation into Blood</th>
<th>Nucleated Erythroid Cells × 10^9/mg. Marrow</th>
<th>Per cent Reticulocytes per Thousand Nucleated Marrow Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>55.6 ± 5.2 †</td>
<td>401 ± 61</td>
<td>59.5 ± 0.8</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>14.8 ± 1.6</td>
<td>115 ± 27</td>
<td>50.0 ± 4.7</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>4.7 ± 0.8</td>
<td>287 ± 45</td>
<td>26.3 ± 5.1</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>2.5 ± 0.5</td>
<td>181 ± 30</td>
<td>7.8 ± 2.3</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>not done</td>
<td>60 ± 17</td>
<td>not done</td>
</tr>
<tr>
<td>Normal</td>
<td>9</td>
<td>26.0 ± 2.2</td>
<td>497 ± 47</td>
<td>31.1 ± 3.2</td>
</tr>
</tbody>
</table>

* From data collected previously by Turner et al.2
† Days after removal from the decompression chamber.
‡ ± 1 standard deviation.

Table 2.—Formation of Spleen Colonies in Lethally Irradiated Recipients by Marrow Grafts from Donors at Various Stages of Post-Hypoxic Polycythemia (‘Rebound’)

<table>
<thead>
<tr>
<th>Donors Day Rebound</th>
<th>Graft (10^9 Nucleated Marrow Cells)</th>
<th>Spleen Colonies</th>
<th>Status of Donor Marrow During Rebound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per Cent Small Lymphocytes</td>
<td>Per Cent Transitional Cells</td>
<td>Per 10^9 Grafted Marrow Cells</td>
</tr>
<tr>
<td>1</td>
<td>15.2 ± 0.1</td>
<td>3.3 ± 1.6</td>
<td>9.2 ± 2.6</td>
</tr>
<tr>
<td>3</td>
<td>19.9 ± 1.0</td>
<td>5.8 ± 1.4</td>
<td>10.9 ± 0.4</td>
</tr>
<tr>
<td>5</td>
<td>25.5 ± 2.6</td>
<td>12.0 ± 0.8</td>
<td>17.2 ± 1.5</td>
</tr>
<tr>
<td>7</td>
<td>37.7 ± 2.9</td>
<td>12.8 ± 0.4</td>
<td>17.4 ± 1.1</td>
</tr>
<tr>
<td>9</td>
<td>30.6 ± 2.9</td>
<td>7.4 ± 0.7</td>
<td>14.9 ± 1.5</td>
</tr>
<tr>
<td>Normal</td>
<td>24.9 ± 1.7</td>
<td>4.3 ± 0.5</td>
<td>8.8 ± 1.4</td>
</tr>
</tbody>
</table>

‡ ± 1 standard deviation.
* Total nucleated cell values obtained in a parallel experiment.2
† Total colony-forming cell and total lymphocytes/mg. of marrow were calculated for each group from the total nucleated cell values and the number of observed spleen colonies or per cent lymphocyte content of the grafts, respectively.

DISCUSSION

The data show that the stem cell content of mouse bone marrow during post-hypoxic polycythemia, as measured by its spleen colony-forming ability, is proportional to its lymphocyte content. This is essentially a confirmation in vivo of Cudkowicz’s finding that in vitro concentration of marrow lymphocytes by glass wool filtration, resulted in a proportional increase in its ability to promote splenic 125I-UdR uptake,3 59Fe uptake, and colony formation.7 On the other hand, using a sedimentation method to concentrate marrow fractions, Mel and Schooley found no correlation between "small, round mononuclear" cell content of mouse marrow grafts and their spleen colony-forming ability.8 Schooley suggested later, that although erythropoietin sensitivity seems to be
related to the number of marrow lymphocytes, colony-forming ability might be attributed to either a very small proportion of marrow lymphocyte-like cells or to an altogether different morphologic category of cells.\textsuperscript{5}

Although our findings are not wholly in agreement with Schooley's, they do seem to be in agreement with one of his propositions, i.e., that colony-forming cells might constitute a small proportion of marrow lymphocytes. While these data suggest that a constant proportion of bone marrow lymphocytes can form spleen colonies, the number of lymphocytes contained in the grafts exceeded the actual number of colonies formed by a factor of at least $2 \times 10^9$. Even if it is taken into account that only a small per cent of the CFU's contained in a graft colonize the spleen,\textsuperscript{9} it is evident that if lymphocytes possess this ability, only a small proportion actually do so. A comparison of the relative number of transitional cells and small lymphocytes contained in marrow grafts and the number of spleen colonies formed (Table 2) reveals that the number of transitional cells more closely correlates with colony-forming cell content than does the number of small lymphocytes. The actual number of transitional cells grafted was only a small percentage of the total lymphocyte content of the graft.

It has been reported that during regeneration of the stem cell compartment following irradiation, a large fraction of the colony-forming cells are susceptible to the lethal effects of exposure to high specific activity $^3$HTdR, while in the normal steady state most of the colony-forming cells are not affected by 'suicidal' quantities of the isotope.\textsuperscript{10} During post-hypoxic polycythemia,
the colony-forming cell population is either regenerating or accumulating. Also during this time, there is a corresponding increase in the total lymphocyte population, and in particular, in the transitional cells. Transitional cells have been shown to be the only lymphocyte-like cells capable of immediate incorporation of $^3$HTdR, and to be precursors to small lymphocytes as well as to blast cells. Therefore, transitional cells are the more feasible candidates for colony-forming cells during enlargement of the hemopoietic precursor population, while some of their non-DNA synthesizing counterparts might preserve this function during the normal, steady state.

In conclusion, the correlation between the increased colony-forming ability and lymphocyte accumulation accompanying the erythropoietic depression following temporary exposure to hypoxia is intriguing. Further work in this area may help to elucidate the interrelationship between hemopoietic demand and the size of the stem cell population. However, a more direct kind of evidence will be required before it can be assumed with certainty that bone marrow lymphocytes or transitional cells participate in hemopoiesis. These studies are being continued in order to determine whether the increased erythropoietin sensitivity of polycythemic animals can be related to the increased colony-forming ability and lymphocyte content of their marrows.

Summary

As assessed by its spleen colony-forming ability, the stem cell content of bone marrow from mice recovering from hypoxia increases with the duration of erythropoietic depression, and is directly proportional to the number of marrow lymphocytes.

SUMMARIO IN INTERLINGUA

A base del observate capacitate de formar colonias splenic del contento de cellulas primordial de medulla ossee in muses in stato de reconvalescentia ab hypoxia, il pare que iste contento accresce con le duration del depression erythropoietic e es directemente proportional al numero de lymphocytos medullari.

REFERENCES


8. Mel, H. S., and Schooley, J. C.: Stable-


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