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INTRODUCTION

As a result of a genetic defect expressed in the stroma of the tissues supporting hematopoiesis rather than in the hematopoietic cells themselves, mice of genotype Sl/Sld (Steel, Steel-Dickie mutant mice) suffer a chronic macrocytic anemia, and are extremely sensitive to ionizing radiation (11). Previously, it was hypothesized that the genetic defect disturbs erythropoiesis very early in the erythron, perhaps at the point of commitment of in vivo colony-forming units (CFU) to the erythrocytic cellular line of differentiation (8, 19). In an earlier study (9), this hypothesis was tested by measuring and comparing, in Sl/Sld mice and their congenic +/- littermates, population sizes of high self-renewal potential and low self-renewal potential CFU. It was reasoned that a block in stem cell differentiation occurring early in the erythron would result in a deficiency of the latter but not of the former. However, our study did not bear this hypothesis out. Rather, it led to the unexpected observation that all the stem cell populations in Sl/Sld mice, with the exception of the splenic CFU population, were reduced in size.

However, anemia and other forms of hematopoietic stress are known to initiate substantial increases in extramedullary, but not medullary CFU population sizes (7, 10), and, because Sl/Sld mice suffer a chronic macrocytic anemia, it was reasoned that a comparison of the effects of Sl and + genes on CFU population sizes might be more meaningful if the comparison were undertaken not only on the same genetic background but also under similar physiologic conditions in which the blood RBC concentrations of +/- and Sl/Sld mice are approximately the same. Therefore, in the present study, anemic Sl/Sld mice and normal +/- mice were rendered polycythemic by hypertransfusion and the sizes of their CFU population determined.

METHODS

MICE

WCB6F, Sl/Sld, B6D2F1, C57BL/6J, and WC/Re mice were obtained from the Jackson Laboratory, Bar Harbor, Me. B6WCF1 mice were raised at the Armed Forces Radiobiology Research Institute by mating C57BL/6J females with WC/Re males. The animals were maintained on a 6 AM to 6 PM (light-dark) cycle. Wayne Lab-Blox and acidified (pH 2.5) water were available ad libitum. All mice were acclimated to laboratory
Humoral and Cellular Control Agents

conditions for 2 weeks. During this time they were certified free of lesions of murine pneumonia complex and of oropharyngeal *Pseudomonas* spr.

RADIATION

Mice were exposed to $^{60}$Co wholebody gamma-radiation at a dose rate of 150 rad/min to a total dose of 950 rad.

**IN VIVO COLONY FORMING ASSAY (CFU)**

The CFU assay of Till and McCulloch (16) was performed as previously described (9). The following donor-recipient combinations were used: B6D2F1 hematopoietic cells were transplanted into B6D2F1 mice and WCB6F1/S/S into B6WCF1 mice.

**CFU SEEDING EFFICIENCY**

The 2-hour seeding efficiencies $f$ of CFU were determined according to the method of Siminovitch et al. (14). Briefly, in the case of femoral CFU, $6 \times 10^8$ marrow cells from a pool of one to four donor mice were injected into five intermediate recipients. Two hr later the mice were euthanized, their spleens removed, and 1/16 to 1/12 of a spleen was then injected into 10 secondary recipients. In the case of splenic CFU, 1–5 $\times 10^8$ spleen cells from a pool of one to four donor mice were injected into five intermediate recipients. Two hours later 0.25–0.5 of a spleen was injected into four secondary recipients. The CFU content of the original cell suspensions was determined in primary recipients by the *in vivo* CFU assay.

**HYPERTHERFUSION**

Blood for transfusion was collected from normal and heterozygous littermates from the orbital sinus into heparinized phosphate buffered saline and washed 3 times. One-half ml of washed packed red cells was then injected i.p. into each recipient daily on 3 successive days. Six days after the last injection, hematocrit (Hct.) values of the peripheral blood were determined. Mice having a Hct. of at least 55 were considered hypertransfused.

**CALCULATIONS**

CFU per $10^8$ cells was calculated according to the formula: CFU/$10^8$ cells = observed colonies/$10^8$ cells $\times$ $1/f$. CFU per organ was calculated according to the formula: CFU/organ = CFU/cell $\times$ cells/organ.

**RESULTS**

**ORGAN CELLULARITY AND CFU NUMBERS IN NORMAL AND HYPERTHERFUSED +/+ MICE**

Presented in Table 1 and Table 2 are the number and colony-forming potential of nucleated cells from the spleen and femurs respectively of normal and hypertransfused male B6D2F1 of genotype +/+ . It was found, as has been

**TABLE 1** Colony-Forming Ability of Spleen Cells from Normal and Hypertransfused B6D2F1, +/+ Mice

<table>
<thead>
<tr>
<th>Hct. range</th>
<th>48–50</th>
<th>67–76</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonies/$10^8$ cells</td>
<td>2.85 ± 0.73*</td>
<td>3.60 ± 0.51</td>
</tr>
<tr>
<td>[4]</td>
<td>[3]</td>
<td></td>
</tr>
<tr>
<td>Nucleated cells/spleen ($\times 10^9$)</td>
<td>6.35 ± 0.85</td>
<td>8.35 ± 2.32</td>
</tr>
<tr>
<td>[4]</td>
<td>[3]</td>
<td></td>
</tr>
<tr>
<td>f (%)</td>
<td>13.6 ± 3.1</td>
<td>4.2 ± 1.2</td>
</tr>
<tr>
<td>[4]</td>
<td>[3]</td>
<td></td>
</tr>
<tr>
<td>CFU/$10^8$ cells</td>
<td>19</td>
<td>85</td>
</tr>
<tr>
<td>CFU/spleen $^a$</td>
<td>12,200</td>
<td>71,500</td>
</tr>
</tbody>
</table>

*Mean ± SE.

$^a$Figures in brackets refer to number of separate determinations. Each determination consisted of a cell suspension prepared from three to four experimental mice being injected into seven to twelve recipients.

$^b$Calculated from data for $f$ and colonies/$10^9$ cells.

$^c$Calculated from CFU/$10^8$ cells and average number of cells per donor spleen.

**TABLE 2** Colony-Forming Ability of Marrow Cells from Normal and Hypertransfused B6D2F1, +/+ Mice

<table>
<thead>
<tr>
<th>Hct. range</th>
<th>48–50</th>
<th>67–76</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonies/$10^8$ cells</td>
<td>20</td>
<td>58</td>
</tr>
<tr>
<td>[1]</td>
<td>[1]</td>
<td></td>
</tr>
<tr>
<td>Nucleated cells/femur ($\times 10^9$)</td>
<td>1.07</td>
<td>1.13</td>
</tr>
<tr>
<td>[1]</td>
<td>[1]</td>
<td></td>
</tr>
<tr>
<td>f (%)</td>
<td>17.5</td>
<td>8.2</td>
</tr>
<tr>
<td>[1]</td>
<td>[1]</td>
<td></td>
</tr>
<tr>
<td>CFU/$10^8$ cells</td>
<td>115</td>
<td>700</td>
</tr>
<tr>
<td>CFU/femur $^a$</td>
<td>11,800</td>
<td>75,000</td>
</tr>
</tbody>
</table>

*Figures in brackets refer to number of separate determinations. Each determination consisted of a cell suspension prepared from four experimental mice being injected into twelve recipients.

$^a$Calculated from data for $f$ and colonies/$10^8$ cells.

$^b$Calculated from CFU/$10^8$ cells and average number of cells per donor marrow.
reported by others (3, 13), that hypertransfusion increases the colony-forming potential of hematopoietic nucleated cells from both the marrow and spleen. Also, it was found that hypertransfusion decreases the CFU seeding efficiency 2–4-fold. Therefore, correcting for changes in both the spleen colony-forming potential and f, it was calculated that the femoral and splenic CFU population sizes of hypertransfused mice are approximately 4–6-fold larger on either a per cellular or per organ basis than are comparable CFU population sizes in normal mice.

**ORGAN CELLULARITY AND CFU NUMBERS IN NORMAL AND HYPERTRANSFUSED SI/Sl" MICE**

As compared to male mice of genotype +/+, hypertransfusion has exactly the opposite effect on the CFU populations of male SI/SI" mice. Hypertransfusion (a) lowers the colony-forming potentials of the hematopoietic nucleated cells rather than increasing them; and (b) increases rather than decreases f (Tables 3 and 4). Taking these differences into consideration when calculating the CFU population sizes of hypertransfused SI/SI" mice, as compared to anemic SI/SI" mice, it was determined that hypertransfusion drastically reduces by about 50-fold the size of the splenic CFU population, and to a lesser extent—about 2-fold—the size of the marrow CFU population.

**DISCUSSION**

It was the tentative conclusion of a previous study (9) that the factors supporting a normal size splenic CFU population in SI/SI" mice were predominantly long-range or systemic in nature, and were produced in response to the macrocytic anemia suffered by these mice (5). This hypothesis was tested in the present study by temporarily eliminating the anemia of SI/SI" mice by hypertransfusion and measuring their CFU population sizes. It was found that hypertransfusion reduced the SI/SI" splenic CFU population size 50-fold while reducing that of the marrow only 2-fold. In contrast, this same treatment increased both marrow and splenic CFU numbers of +/+ mice 6-fold.

When these findings are viewed in the light of the recent work of Bozzini et al. (1), they offer an insight into the puzzling fact that although erythropoiesis in SI/SI" mice is erythropoietin-dependent (12), polycyhemic SI/SI" mice are refractory to exogenous erythropoietin (6). Bozzini and coworkers clearly demonstrated that the early reestablishment of erythropoiesis in polycytemic +/+ mice by exogenous erythropoietin is nearly in toto a splenic phenomenon. Therefore, given the stem cell-progenitor cell relationship of CFU to erythropoietin-responsive cells (ERC), the lack of a splenic erythropoietin-responsive compartment in hypertransfused SI/SI" mice is consistent with the present finding of a nearly total absence of splenic CFU in these mice. As such, it might be concluded that the refractory character of hypertransfused SI/SI" mice to exogenous erythropoietin is due to the absence of a splenic compartment that is responsive to erythropoietin.

**TABLE 3** Colony-Forming Ability of Spleen Cells from Normal and Hypertransfused WC66F1 – SI/SI" Mice

<table>
<thead>
<tr>
<th>Hct range</th>
<th>22–33</th>
<th>55–66</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonies/10^5 cells</td>
<td>2.60 ± 0.82*</td>
<td>0.27 ± 0.12</td>
</tr>
<tr>
<td>Nucleated cells/ spleen (× 10^4)</td>
<td>1.72 ± 0.21</td>
<td>1.34 ± 0.16</td>
</tr>
<tr>
<td>f (%)</td>
<td>4.4</td>
<td>14.7 ± 12.0</td>
</tr>
<tr>
<td>CFU/10^6 cells</td>
<td>59.1</td>
<td>1.8</td>
</tr>
<tr>
<td>CFU/spleen</td>
<td>102,000</td>
<td>2460</td>
</tr>
</tbody>
</table>

*Mean ± SE.
*Figures in brackets refer to number of separate determinations. Each determination consisted of a cell suspension prepared from one to two experimental mice being injected into seven to twelve recipient mice.
*Calculated from data for f and colonies/10^6 cells.
*Calculated from CFU/10^6 cells and average number of cells per donor spleen.

**TABLE 4** Colony-Forming Ability of Marrow Cells from Normal and Hypertransfused WC66F1 – SI/SI" Mice

<table>
<thead>
<tr>
<th>Hct range</th>
<th>22–33</th>
<th>55–66</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonies/10^5 cells</td>
<td>15.00 ± 3.50*</td>
<td>12.35 ± 1.35</td>
</tr>
<tr>
<td>Nucleated cells/ femur (× 10^4)</td>
<td>1.95 ± 0.16</td>
<td>1.51 ± 0.09</td>
</tr>
<tr>
<td>f (%)</td>
<td>7.3</td>
<td>15.0</td>
</tr>
<tr>
<td>CFU/10^6 cells</td>
<td>185</td>
<td>82</td>
</tr>
<tr>
<td>CFU/femur</td>
<td>20,200*</td>
<td>12,400</td>
</tr>
</tbody>
</table>

*Mean ± SE.
*Figures in brackets refer to number of separate determinations. Each determination consisted of a cell suspension prepared from one to two experimental mice being injected into seven to twelve recipient mice.
*Calculated from CFU/10^6 cells and average number of cells per donor femur.
erythropoietin is a result, in part, of anomalous cell kinetics at the stem cell level. In addition to a long-range mechanism regulating CFU proliferations, there is considerable evidence for a local one. For example, it is known that the selective depopulation of a hematopoietic cell maturation compartment, such as the erythron, results in the recruitment of CFU into cell cycle (3, 18). However, in the $S_i/S_i^a$ mouse, suppression of the erythron by hypertransfusion does not appear to stimulate CFU proliferation. This might suggest that the stromal tissues of $S_i/S_i^a$ mice are incapable of producing an effective local CFU proliferative factor in response to a depleted erythron. Indeed, similar observations on the absence of a local CFU proliferative mechanism in $S_i/S_i^a$ mice have been reported by others (4, 15).

Given the concepts that (a) commitment of hematopoietic stem cells to the erythrocytic cellular line of differentiation is regulated by local stromal tissue, i.e., the hematopoietic inductive microenvironment (HIM), and (b) the $S_i$ element is an integral part of this microenvironment (17), it might follow from the present work that the erythrocytic HIM can be described, in part, as a feedback loop between the erythron and the multipotent CFU compartment via the specialized stromal tissues supporting hematopoiesis. The mechanism would operate in such a fashion that CFU proliferation would be stimulated by a stromal tissue-CFU interaction in response to a depleted erythron. The exact nature of this stromal-supported CFU proliferation is, of course, not known. However, it could be speculated that this proliferative mechanism by itself or in unison with other factors generates and/or, amplifies a CFU subpopulation with a high capacity for erythropoiesis that does take place in $S_i/S_i^a$ mice does so at erythropoietin concentrations characteristic of in vitro rather than in vivo systems (2, 6).

**SUMMARY**

The effect of hypertransfusion on the colony-forming unit (CFU) population size of normal and mutant $S_i/S_i^a$ mice was determined. The main finding was that hypertransfusion reduced the splenic CFU population of $S_i/S_i^a$ nearly 50-fold while increasing that of normal mice 6-fold. Hypertransfusion also reduced the marrow CFU population of $S_i/S_i^a$ mice, but the reduction was only 2-fold. In normal mice, hypertransfusion resulted in a 6-fold increase in the marrow CFU population. Two tentative conclusions were drawn from the present study: (a) the refractoriness of the polycythemic $S_i/S_i^a$ mice to exogenous erythropoietin is a result of anomalous stem cell kinetics characterizing the hypertransfused $S_i/S_i^a$ mouse; and (b) the hematopoietic inductive microenvironment can be described, in part, as a feedback loop between the erythron and multipotent CFU compartment via the specialized stromal tissues which support hematopoiesis.

**REFERENCES**


In Vivo Colony Forming Unit Population Sizes in Hypertransfused S/Si² Mice

The effect of hypertransfusion on the colony-forming unit (CFU) population size of normal and mutant S1/Sld mice was determined. The main finding was that hypertransfusion reduced the splenic CFU population of S1/Sld mice nearly 50-fold while increasing that of normal mice 6-fold. Hypertransfusion also reduced the marrow CFU population of S1/Sld mice, but the reduction was only 2-fold. In normal mice, hypertransfusion resulted in a 6-fold increase in the marrow CFU.
20. ABSTRACT (continued)

population. Two tentative conclusions were drawn from the present study: (a) the refractoriness of the polycythemic Sl/Sl<sup>d</sup> mice to exogenous erythropoietin is a result of anomalous stem cell kinetics characterizing the hypertransfused Sl/Sl<sup>d</sup> mouse; and (b) the hematopoietic inductive microenvironment can be described, in part, as a feedback loop between the erythron and multipotent CFU compartment via the specialized stromal tissues which support hematopoiesis.