Abnormalities of Megakaryocytes in Sl/Sl^d Mice

By Shirley Ebbe, Elizabeth Phalen, and Frederick Stohlman, Jr.

Megakaryocytopoiesis was evaluated in Sl/Sl^d mice and their heterozygous and homozygous normal (+/+) littermates. Sl/Sl^d mice had a normal concentration of blood platelets which were of normal size. Numbers of megakaryocytes in bone marrow and spleen were reduced, but individual megakaryocytes were larger than normal.

The microenvironment has been shown to influence hematopoietic cell differentiation into erythropoietic or granulocytopoietic cell systems. Megakaryocytes are thought to originate from the same pluripotential stem cell as red cells and granulocytes, but nothing is known about micro-environmental influences on megakaryocytopoiesis except that it may be influenced by body age.

The Sl/Sl^d mouse has a genetically determined macrocytic anemia that appears to be due to a defect in the cellular environment rather than to defective hematopoietic cells. The anemia of Sl/Sl^d mice is not corrected when normal hematopoietic stem cells are transplanted into them, but it is improved by transplantation of intact spleens from normal donors. SI/Sl^d spleens have, further, been shown to be incapable of supporting colony formation by transplanted hematopoietic stem cells in irradiated hosts. The W/W^v mouse also has hereditary macrocytic anemia associated with a defect in transplantable stem cells. The dependence of the anemia on the stem-cell defect in W/W^v and on the tissue-environmental defect in SI/Sl^d is shown by the facts that transplantation of stem cells from SI/Sl^d to W/W^v will cure the anemia in the W/W^v, but splenic tissue transplantation, rather than just stem-cell transplantation, from W/W^v to SI/Sl^d is necessary to improve the anemia of the SI/Sl^d.

Sutherland et al. found that granulocytopoiesis was not totally normal in Sl/Sl^d mice. However, the effect of the SI defect on megakaryocytopoiesis has not been reported; hence, the present studies were undertaken.

MATERIALS AND METHODS

Female mice of the SI/Sl^d strain and their heterozygous and homozygous +/+ female littermates were obtained from the Jackson Laboratories, Bar Harbor, Me. Experiments were done when the mice were 12–14 wk of age. Body weights were determined on 42 mice with the following...
Table 1. Hematocrits, Mean Red Cell Volumes, and Reticulocytes for Various SL Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Hematocrit (%</th>
<th>MCV (cu μ)</th>
<th>Reticulocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI/Sld</td>
<td>27.8 ± 0.91*</td>
<td>77.0 ± 2.13</td>
<td>2.8 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>(20)†</td>
<td>(9)</td>
<td>(9)</td>
</tr>
<tr>
<td>SI/+</td>
<td>42.7 ± 1.23</td>
<td>51.1 ± 1.77</td>
<td>1.3 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>Sld/+</td>
<td>41.7 ± 1.47</td>
<td>49.9 ± 1.60</td>
<td>1.0 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(8)</td>
<td>(8)</td>
</tr>
<tr>
<td>++</td>
<td>42.4 ± 1.49</td>
<td>46.3 ± 0.84</td>
<td>0.8 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(8)</td>
<td>(8)</td>
</tr>
</tbody>
</table>

*Average ± SEM.
†Number of mice in parenthesis.

results: 13 SI/Sld had a mean weight of 21.2 g (range 17-26 g), 7 SI/+ averaged 23.9 g (20.5-27 g), 10 SId/+ averaged 24.4 g (22.9-27 g), and 12 ++ averaged 23.1 g (20-27 g).

Animal maintenance and experimental procedures were exactly the same as described in an accompanying paper. 10

RESULTS

Anemia

Table 1 lists hematocrits, mean red cell volumes, and reticulocytes for the various SI genotypes. Only the SI/Sld showed macrocytic anemia and relative reticulocytosis.

Thrombocytopoiesis, SI/Sld

Counts of platelets and megakaryocytes in the SI/Sld mice, their normal, and heterozygous littermates are shown in Table 2. Platelet counts in the four groups were the same. In the tibiae of SI/Sld mice the numbers of both megakaryocytes and total nucleated cells were significantly (p < 0.001) reduced below those of ++ controls. The percentage of megakaryocytes was not significantly different (p > 0.05), indicating that megakaryocytes were reduced by about the same amount as other nucleated cells. In the spleen, megakaryocytes were reduced to about half the control value not only in SI/Sld mice, but also in their heterozygous littermates; all of these reductions were significant (p < 0.001).

Table 2. Platelet and Megakaryocyte Counts

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Blood Platelets/cu mm (x 10¹¹)</th>
<th>Megakaryocytes (x 10⁴)</th>
<th>Nucleated Cells (x 10⁴)</th>
<th>Per Cent Megakaryocytes</th>
<th>Spleen Megakaryocytes Per Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI/Sld</td>
<td>1.389 ± 0.074*</td>
<td>4.8 ± 0.6</td>
<td>8.590 ± 0.431</td>
<td>0.055</td>
<td>56.5 ± 10.63</td>
</tr>
<tr>
<td></td>
<td>(20)†</td>
<td>(16)</td>
<td>(16)</td>
<td></td>
<td>(15)</td>
</tr>
<tr>
<td>SI/+</td>
<td>1.294 ± 0.076</td>
<td>10.8 ± 1.5</td>
<td>14.545 ± 0.621</td>
<td>0.074</td>
<td>58.0 ± 4.81</td>
</tr>
<tr>
<td></td>
<td>(12)</td>
<td>(10)</td>
<td>(10)</td>
<td></td>
<td>(10)</td>
</tr>
<tr>
<td>Sld/+</td>
<td>1.391 ± 0.077</td>
<td>8.8 ± 0.7</td>
<td>13.904 ± 0.561</td>
<td>0.064</td>
<td>49.2 ± 6.63</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(14)</td>
<td>(14)</td>
<td></td>
<td>(14)</td>
</tr>
<tr>
<td>++</td>
<td>1.268 ± 0.042</td>
<td>10.5 ± 0.9</td>
<td>15.137 ± 0.380</td>
<td>0.069</td>
<td>115.8 ± 5.56</td>
</tr>
<tr>
<td></td>
<td>(18)</td>
<td>(15)</td>
<td>(15)</td>
<td></td>
<td>(15)</td>
</tr>
</tbody>
</table>

*Average ± SEM.
†Number of mice in parenthesis.
ABNORMALITIES OF MEGAKARYOCYTES IN SI/SI<sup>d</sup> MICE

Fig. 1. Size distribution curves for mature megakaryocytes from SI/SI<sup>d</sup> mice and their +/+ controls. Size is expressed in planimeter units.

From these data, it appeared that SI/SI<sup>d</sup> were maintaining a normal number of circulating platelets with only a half-normal complement of megakaryocytes. Size distribution curves of granular, mature, stage-III megakaryocytes (Fig. 1) showed that megakaryocyte mass was not as low in the SI/SI<sup>d</sup> mice as would be

Table 3. Analysis of Megakaryocyte Size Distribution Curves

<table>
<thead>
<tr>
<th>Stage&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Genotype</th>
<th>Average Size (Planimeter Units)</th>
<th>Value ± SEM</th>
<th>Significance of difference From +/+ (Student's t-test)</th>
<th>χ² Analysis (Probability of Identity With +/+)&lt;sup&gt;†&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>SI/SI&lt;sup&gt;d&lt;/sup&gt; (16)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>1332.4 ± 21.2 (522)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>III</td>
<td>SI/+ (10)</td>
<td>946.2 ± 20.9 (347)</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.05, &gt; 0.02</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>SI&lt;sup&gt;d&lt;/sup&gt;/+ (14)</td>
<td>869.0 ± 16.5 (479)</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>+/+ (15)</td>
<td>848.2 ± 16.6 (525)</td>
<td>p &gt; 0.30</td>
<td>p &gt; 0.80</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>SI/SI&lt;sup&gt;d&lt;/sup&gt; (16)</td>
<td>735.4 ± 53.0 (47)</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>+/+ (8)</td>
<td>420.7 ± 11.4 (170)</td>
<td>p &gt; 0.30</td>
<td>p &gt; 0.80</td>
<td></td>
</tr>
</tbody>
</table>

<sup>*</sup>Stage of megakaryocyte maturation (III is mature, I is immature).
<sup>†</sup>Number of mice.
<sup>‡</sup>Number of megakaryocytes.
suggested by the cell counts alone. Megakaryocytes from their bone marrow showed a prominent shift in the distribution curve toward the larger sizes, the average size of stage-III $\text{Sl/Sld}^d$ megakaryocytes being $157\%$ that of $+/+$. Size distribution curves of stage-III megakaryocytes from heterozygotes were comparable to those from controls. Splenic megakaryocytes were not sized, but they were obviously larger in $\text{Sl/Sld}^d$ than in any of their littermates. Table 3 shows average sizes of megakaryocytes from the four genotypes. The Student’s $t$ test indicated that the average size of stage-III megakaryocytes was significantly different from normal in both $\text{Sl/Sld}^d$ mice and $\text{Sl}^d/+$ heterozygotes. However, the size distribution curves showed a tendency to be skewed toward larger sizes, and when each curve was tested by the $\chi^2$ test for identity with the population of megakaryocytes from $+/+$ mice, only the curve of $\text{Sl/Sld}^d$ megakaryocytes was significantly different.

Analysis of size distribution curves of immature stage-I megakaryocytes (basophilic, nongranular cytoplasm) revealed that they, too, were larger in $\text{Sl/Sld}^d$ than the same type of cells in controls, the average size being $175\%$ that of $+/+$. Due to the paucity of megakaryocytes, this determination was based on 47 stage-I megakaryocytes from 16 $\text{Sl/Sld}^d$ mice and 170 from eight $+/+$ mice. Analysis by both the Student’s $t$ test for difference in mean size and the $\chi^2$ test for difference in distribution indicated a significant difference.
Platelets in Sl/Sld mice did not reflect the increased megakaryocyte size and, in fact, appeared to be slightly smaller than normal (Fig. 2). Average platelet size of Sl/Sld mice was 88%, that of their controls. Platelet size in Sld/+ mice was comparable to that of Sl/Sld, while Sl/+ mice showed the same curve as the +/+ controls. However, χ² analysis indicated that none of the platelet size distribution curves differed significantly from normal.

DISCUSSION

Mice of the Sl/Sld strain had normal platelet counts, and platelet size was normal. Hence, they had a normal platelet mass per unit of blood. However, megakaryocyte numbers in tibial marrow and spleen were reduced to about half of normal, and the megakaryocytes were considerably larger than normal. Megakaryocytes of both early and late stages of maturation were demonstrated to be macrocytic, and in this respect, the macrocytosis resembled that seen in response to the thrombocytopenia stimulation of peripheral platelet depletion. The fact that circulating platelets were small rather than large suggests that they were not produced in response to the same type of thrombocytopenic stimulation as is produced by acute thrombocytopenia. If there is stimulation of megakaryocytosis, its origin is unclear in the absence of peripheral thrombocytopenia. It could be proposed that there are internal regulatory mechanisms by which paucity of cells stimulates nuclear proliferation of precursor cells, but it could equally well be proposed that the increased individual cell mass imposed by intrinsic macrocytosis may inhibit cellular proliferation, thereby leading to reduced numbers. Alternatively, megakaryocyte size and number may not be related to one another by intramedullary mechanisms. Jackson et al. have recently reported evidence to suggest that platelet production may be stimulated by erythropoietin if erythropoiesis is inhibited. Sl/Sld and W/Wv mice both have endogenous erythropoietin, but their erythropoietic systems do not respond normally to erythropoietin. Thus, the mechanism proposed by Jackson may explain, in part, the thrombocytopenic abnormalities in these mice, even though erythropoietin does not appear to affect platelet production in the normal animal.

Odell et al. have shown that the size of megakaryocytes in normal animals is proportional to nuclear ploidy, and Penington and Olsen demonstrated that the changes that occur in megakaryocyte size when this cell system is stimulated or suppressed are accompanied by changes in nuclear ploidy. Therefore, it is probable that the macromegakaryocytes in Sl/Sld mice have more DNA than cells of normal size. The average size of stage III megakaryocytes in Sl/Sld mice was about 1.6 times normal, suggesting that the population as a whole may have had nearly twice the normal amount of DNA, as Odell et al. found that doubling the amount of DNA in mature megakaryocytes was associated with an increase in total cell size of 1.7-1.8 times. Harker has proposed that cytoplasmic mass increases proportionately with nuclear ploidy, and that the larger megakaryocyte, the more platelets it produces. The nuclear/cytoplasmic ratio was not measured in Sl/Sld mice, but if the macromegakaryocytes had an increased cytoplasmic volume, then the macrocytosis may have compensated, in part, for reduction in megakaryocyte number. As judged by platelet size
distribution curves, the megakaryocytes were fragmenting into platelets of normal size, so each large megakaryocyte may have been producing more platelets than normal.

Odell\textsuperscript{18} has recently speculated that the hematopoietic microenvironment may play a role in determining when megakaryocytes cease to replicate DNA and hence determine the ploidy level at which cytoplasmic maturation and platelet formation occur. The observations of others that were summarized in the introduction clearly indicate that SI/SI\textsuperscript{d} mice have a genetically determined abnormality of the hematopoietic microenvironment. The cellular or chemical nature of this abnormality has not been defined, but it has been reported that the splenic stroma of SI/SI\textsuperscript{d} mice have an increased concentration of sulfated acid mucopolysaccharide.\textsuperscript{19} The present observations that show abnormalities of megakaryocyte size and are suggestive of abnormalities of ploidy provide support for, but not proof of, Odell's hypothesis.

The reduced numbers of splenic megakaryocytes in the SI heterozygotes suggested that this genetic abnormality may affect the spleen more than the marrow. The heterozygotes did not have macromegakaryocytosis, so megakaryocyte mass appeared to be about half of normal in the spleen and normal in the marrow. W/W\textsuperscript{v} mice also showed a disproportionate reduction in splenic megakaryocytes,\textsuperscript{10} thus raising a question of the importance of splenic megakaryocytes in total body platelet production.

Both W/W\textsuperscript{v} and SI/SI\textsuperscript{d} genotypes have reduced numbers of megakaryocytes and macromegakaryocytosis, but the latter was less pronounced in W/W\textsuperscript{v} and was not demonstrable in immature, stage I megakaryocytes. For this reason, the thrombocytopoietic system appears to behave differently in these two strains of genetically anemic mice.

Even though the abnormalities of megakaryocytopoiesis in SI/SI\textsuperscript{d} mice defy explanation at the present time, these observations show that the state of the hematopoietic microenvironment is important to megakaryocytopoiesis, as it is to erythropoiesis and stem-cell function. Further analysis of these mice may lead to a better understanding of the control of thrombocytopoiesis and, possibly, to some insight into human myeloproliferative disorders in which macromegakaryocytosis occurs but cannot be explained by peripheral thrombocytopenia.\textsuperscript{20,21,22}

REFERENCES


6. Gallagher MT, McGarry MP Trentin JJ: Defect of splenic stroma (hematopoietic induc-
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