Letter to the Editor

Absence of Plasma Erythropoietin in Mice with Anemia Induced by Rauscher Leukemia Virus

A recent article has described underproduction of Ep in response to anemia induced in mice with RLV (2). The present report offers further evidence to support and extend this observation.

Our previous work has involved the study of an anemia-inducing isolate of RLV obtained from BALB/c mice bearing a transplantable leukemia (3) that induces a progressive, fatal anemia with splenomegaly and hepatomegaly resulting from the accumulation of erythroblasts (5). Lymphatic leukemia was not a sequela in this disease which appeared entirely to involve an alteration of the erythron. Plasma from anemic mice was obtained by cardiac puncture at times varying from 3 to 93 days after inoculation with 0.1 ml of undiluted RLV plasma and pooled according to hematocrit, i.e., 10 to 20, 21 to 30, and 31 to 40%. These were compared with plasma pools from uninoculated controls with hematocrits approximating 45 to 50%. Separate measurements of erythropoietic activity were made on 3 different pools to preclude possible toxic effects of the plasmas obtained from donor anemic mice. The erythropoietic bioassay was based on $^{59}$Fe incorporation into peripheral RBC of exhypoxic polycythemic CF-1 female mice as described by Camiscoli and Gordon (1). All plasma pools obtained from normal and RLV-infected mice contained <0.05 i.u. of Ep per 0.5 ml as determined by comparison with calibrated secondary standards (Table 1). Before these results were considered meaningful, it was first necessary to show that there was a true reduction in red cell mass rather than merely hemodilution (not included in Ref. 2) and that the cachectic state of severely anemic mice was not in itself the reason for lack of Ep production. Accordingly, we established that a true reduction of red cell mass occurred even in mice made moderately anemic with RLV by measuring dilution of $^{59}$Fe-labeled homologous erythrocytes from normal BALB/c donors. The results showed that the total blood volume was similar in both normal and anemic mice; thus a decreased hematocrit could be explained only on the basis of reduced red cell mass (Table 2).

Table 1

<table>
<thead>
<tr>
<th>Hematocrit range of RLV donor mice plasma pool</th>
<th>% RBC-$^{59}$Fe incorporated in recipient mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test plasma 10-20</td>
<td>2.01 ± 0.27 $^b$</td>
</tr>
<tr>
<td>21-30</td>
<td>2.96 ± 1.47</td>
</tr>
<tr>
<td>31-40</td>
<td>2.65 ± 0.38</td>
</tr>
<tr>
<td>41-50</td>
<td>4.58 ± 0.83</td>
</tr>
<tr>
<td>Control plasma</td>
<td>1.12 ± 0.12</td>
</tr>
<tr>
<td>Standards</td>
<td></td>
</tr>
<tr>
<td>0.9% NaCl solution</td>
<td>0.97 ± 0.08</td>
</tr>
<tr>
<td>0.05 IRP U Ep 3A $^c$</td>
<td>7.71 ± 0.55</td>
</tr>
<tr>
<td>0.20 IRP U Ep 3A</td>
<td>16.83 ± 1.04</td>
</tr>
<tr>
<td>0.80 IRP U Ep 3A</td>
<td>21.71 ± 1.63</td>
</tr>
</tbody>
</table>

$^a$ All test plasmas were administered i.p. in single doses of 0.5 ml.
$^b$ Mean for 4 test mice ± S.E.
$^c$ Step III sheep plasma Ep, Armour Lot K 147 192A, originally supplied by the Hematology Study Section, NIH, USPHS.

Similar blood volumes were also indicated by comparison of total plasma protein determinations (4) from normal and anemic mice which were almost identical.

A second control required to establish the validity of Ep underproduction involved starving mice for 8 days and then treating them with phenylhydrazine 4 to 8 days after initiation of fasting in order to simulate both cachexia and anemia of terminal RLV-infected BALB/c mice. The mean RBC-$^{59}$Fe incorporation level in bioassay recipients for the pooled plasmas from fasted, phenylhydrazine-treated donors was 44.45 ± 1.62, demonstrating extremely high levels of plasma Ep when compared with calibrated Ep standards (Table 1). Previous studies have also shown that normal BALB/c mice from our colony are efficient producers of Ep when anemia

1Supported by USPHS Grants 5-R01-HL03357-15 and 1-R01-CA12815-01 and a grant from the University of Connecticut Research Foundation.

2The abbreviations used are: Ep, erythropoietin; RLV, Rauscher leukemia virus.

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Table 2
Blood volume and red cell mass determinations in BALB/c mice inoculated with RLV, and in uninoculated controls

<table>
<thead>
<tr>
<th>RBC volume</th>
<th>Hemoglobin</th>
<th>Hematocrit</th>
<th>Body wt (g)</th>
<th>Blood volume&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RBC volume&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Plasma protein (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated BALB/c</td>
<td>9.0 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.1 ± 0.34</td>
<td>51.6 ± 0.68</td>
<td>23.1 ± 0.66</td>
<td>6.54 ± 0.33</td>
<td>3.36 ± 0.13</td>
</tr>
<tr>
<td>RLV-inoculated BALB/c</td>
<td>6.2 ± 0.42</td>
<td>11.3 ± 0.78</td>
<td>37.0 ± 2.71</td>
<td>28.8 ± 0.66</td>
<td>6.28 ± 0.24</td>
<td>2.3 ± 0.10</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as ml/100 g body weight as measured by dilution of <sup>55</sup>Fe-labeled BALB/c homologous erythrocytes.

<sup>b</sup> Mean ± S.E. of 5 mice.

was induced by phenylhydrazine. This response, however, seems to be abolished in RLV-infected mice.

The report by Ebert et al. (2) and our findings appear to establish that BALB/c mice infected with RLV are unable to respond to true anemia by increasing Ep production although detectable low levels of the hormone, in mice with extremely low hematocrits, show that complete cessation of Ep production has not occurred. The isolate of RLV that we used does not seem to induce reticulocytosis or hemolytic anemia. Ebert et al. indicate that this is a characteristic response induced by their isolate; however, only a moderate absolute reticulocytosis is shown in Ebert's report (no data on hemolysis were given). An additional difference between the 2 isolates of RLV is that the disease induced by our isolate is much more protracted; death rarely occurred before the onset of profound anemia (hematocrits of 10 to 20%, 80 to 90 days after inoculation with RLV). The occurrence of massive splenic and hepatic erythroblastosis coupled with absence of compensatory reticulocytosis observed in our earlier studies (3) suggest viral-induced maturation block as one possible mechanism contributing to the development of the severe terminal anemia found in this RLV disease. However, the significance of Ep underproduction during the course of this virally induced erythroid dyscrasia and its possible relation to defective erythroblast maturation remains to be established.

REFERENCES


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