Regulation of Erythropoiesis in the Friend Leukemia Mouse

By SHIGERU SASSA, FUMIMARO TAKAKU AND KIKU NAKAO

Even though the importance of erythropoietin in the regulation of erythropoiesis has been established by a considerable quantity of information during the past decade,1-8 whether or not erythropoietin is the sole regulating mechanism for erythropoiesis has been a subject of controversy. Although it is tempting to believe that erythropoietin production controlled by a feedback system (conducted by the oxygen supply to the tissue) determines red cell formation,9 the hypothesis cannot explain the compensated hemolytic syndrome,10 the continuation of the erythropoiesis in the hypertransfused dog,11 and the erythropoietic effect of hemin12 or hemoglobin.13 Moreover, experimental studies showed that the erythropoiesis in the fetus14 or in the newborn15 is governed by factors other than those seen in the adult.

The Friend leukemia virus, isolated by Friend in 1956,16 has been shown to cause an increased erythropoiesis in the inoculated Swiss mice during their course of development of leukemia.17 It was felt, therefore, that the study of the factors governing the increased erythropoiesis in the Friend leukemia would contribute to the understanding of erythropoiesis in pathological states. In the present work, the hematological changes occurring in the ddO mice after the inoculation of the Friend virus have been followed. Having confirmed the occurrence of erythrocytosis in the infected ddO mice, the nature of this increased erythropoiesis was studied by rendering them polycythemic through transfusion.

Materials and Methods

Transmission of Friend Leukemia

All the animals used in the present study were ddO mice inbred in the National Institute of Infectious Diseases in Tokyo. They were maintained on a diet of the chow made by the Oriental Solid Food Company. The Friend leukemia ddY mice were obtained through the courtesy of Dr. T. Sugimura in the National Cancer Center Research Institute in Tokyo.

Cell-free transfer. A ten percent saline homogenate of the spleen of the affected animals was centrifuged at 12500 g for 40 min. to remove cellular particles. The supernatant was carefully removed and again centrifuged at 105,000 g for one hour. The sediment was brought to a volume equal to that of the supernatant solution by adding 0.9 percent NaCl.

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Table 1.—Cell-free Transfer of Friend Leukemia

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Mice</th>
<th>Weight of Spleen (gm.)</th>
<th>Peripheral nucleated cells (/mm.³)</th>
<th>Reticulocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The supernatant of the centrifuged homogenate of spleen at 12,500 g., for 40 min.</td>
<td>3</td>
<td>0.3</td>
<td>14,150</td>
<td>2.9</td>
</tr>
<tr>
<td>105,000 g., 1 hr. supernatant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>105,000 g., 1 hr. sediment</td>
<td>3</td>
<td>1.5</td>
<td>71,700</td>
<td>10.1</td>
</tr>
</tbody>
</table>

One ml. of each solution was injected into mice intraperitoneally and the mice were sacrificed on the 14th day after injection.

Transfer with supernatant of spleen homogenate. A ten per cent saline homogenate of the spleen from the leukemic mice was centrifuged for 10 min. at 3,000 r.p.m. and 0.2 ml. of the supernatant was injected intraperitoneally. The number of cells in an inoculum was generally about 10⁶.

Red Cell Transfusion to the Friend Leukemia Mice

Seven days after the inoculation of the spleen homogenate, the mice were made polycythemic according to the method described by Gurney et al.¹⁸ On the 14th day after the inoculation, splenic radioiron incorporation was measured as described below.⁴

Determination of Erythropoietic Activity of Plasma and Spleen Extract of Friend Leukemia Mice

Plasma and spleens were collected from the infected mice 14 days after inoculation. Plasma was incubated at 56 C. for 30 min. to inactivate the Friend leukemia virus.¹⁶ Ten and two-tenths grams of spleens collected from eight Friend leukemia mice were homogenized with three volumes of saline in Potter-Elvejhem glass homogenizer. The homogenate was centrifuged at 2,000 r.p.m. for 15 min. and the supernatant was brought to pH 5.5 by adding hydrochloric acid. The acidified supernatant was boiled for five min. according to the method described by Borsook.¹⁹ The heated supernatant was centrifuged at 2,000 r.p.m. for 15 min. To the precipitate, an equal volume of water was added and similarly heated two more times. All the collected supernatant was lyophilized. The final yield from the 10.2 gm. of spleens was 130 mg. One ml. of the plasma and 30 mg. of the heat-extract of the spleen was injected into each polycythemic mouse to assay their erythropoietic activity by the method of Filmanowicz et al.²⁰

Radioiron Incorporation into the Spleen of the Friend Leukemia Mice

One-tenth microcurie of radioiron (⁹⁹FeCl₃) in 0.4 ml. of unbuffered saline solution was injected into the tail vein. Six hours after injection, the mice were sacrificed. The spleen was weighed and an imprint specimen was obtained from the cut surface. It was then thoroughly dissolved in 2 ml. of fuming nitric acid, and radioactivity was determined by a low background well-type scintillation counter.

Hematological Studies of the Mice

Blood was taken from the tail vein. The hematocrit was measured by a microhematocrit capillary tube. The RBC count and the number of peripheral nucleated cells were determined by the use of routine diluting pipettes. Reticulocytes were stained with New Methylene Blue, blood smears and spleen imprints with Wright-Giemsa.

RESULTS

As shown in Table 1, infectivity of Friend virus in ddO mice was demon-
As shown in Fig. 1, reticulocytosis was observed in the infected mice on the seventh day after inoculation and thereafter increased. Increase in hematocrit (Fig. 2) and in red cell count (Fig. 3) was first observed on the 21st day, reaching the maximum on the 35th day. The weight of the spleen began to increase on the seventh day after inoculation, and reached a weight of more than
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Fig. 3.—Increase in red cell count in ddO mice infected with Friend Leukemia Virus.

Fig. 4.—Increase of the spleen weight in ddO mice infected with Friend Leukemia Virus.

ten times normal (Fig. 4). The number of peripheral nucleated cells—consisting mainly of large mononuclear cells, having a round nucleus, and erythroblasts—increased after 14 days, and reached 123,000/mm³ on the sixth week (Fig. 5). Although the spleen continued to increase in weight, the reticulocyte count, hematocrit and red cell count slightly decreased after 35 days.

Microscopic examination of the imprint specimen of the spleen of the mice showed marked infiltration of abnormal cells and of erythroblasts. The abnor-
Fig. 5.—Changes in peripheral nucleated cell count in ddO mice infected with Friend Leukemia Virus.

Fig. 6.—Six hour uptake of $^{59}$FeCl$_3$ in spleens of ddO mice infected with Friend Leukemia Virus.

Mal cells were generally large with dark blue cytoplasm and pale round nuclei of fine chromatin structure. A few nucleoli were observed in the nucleus. Most of the erythroblasts were mature forms with no apparent morphological abnormalities.

Splenic radioiron uptake showed a sharp rise on the seventh day before other manifestations of the disease were detected, and reached 25 percent on the 14th day; while the normal group showed only 4–6 percent (Fig. 6).
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Table 2.—Effect of Transfusion upon the Erythropoiesis in Normal Mice and in Friend Leukemia Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Mice</th>
<th>Ht. (%)</th>
<th>Weight of Spleen (gm.)</th>
<th>Reticulocytes (%)</th>
<th>6 hour uptake of 59Fe by Spleen (%)</th>
<th>Erythroblast in Spleen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5</td>
<td>47</td>
<td>0.12</td>
<td>3.8</td>
<td>6.39</td>
<td>8.3</td>
</tr>
<tr>
<td>Normal transfused</td>
<td>5</td>
<td>67</td>
<td>0.13</td>
<td>0</td>
<td>1.08</td>
<td>0</td>
</tr>
<tr>
<td>Friend</td>
<td>5</td>
<td>50</td>
<td>1.26</td>
<td>13.3</td>
<td>25.0</td>
<td>56.0</td>
</tr>
<tr>
<td>Friend transfused</td>
<td>5</td>
<td>73</td>
<td>1.23</td>
<td>6.3</td>
<td>30.1</td>
<td>41.9</td>
</tr>
</tbody>
</table>

Table 3.—Erythropoietic Activity of the Plasma and the Spleen Extract of Friend Leukemia Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Mice</th>
<th>Ht. (%)</th>
<th>48 hour Uptake of 65Fe by Red Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>70</td>
<td>0.16</td>
</tr>
<tr>
<td>Plasma of Friend mice</td>
<td>3</td>
<td>63</td>
<td>0.09</td>
</tr>
<tr>
<td>Spleen Extract of Friend mice</td>
<td>4</td>
<td>72</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Results of red cell transfusion of the Friend leukemia mice are shown in Table 2. When normal mice were made polycythemic by red cell transfusion, erythropoiesis completely ceased, while the transfusion of Friend leukemia mice had no effect on hematopoiesis. Friend leukemia mice made polycythemic by transfusion showed a similar increased erythropoiesis as the control Friend leukemia mice.

The plasma and the heat-extract of the spleen of Friend leukemia mice showed no erythropoietic activity when assayed with polycythemic mice (Table 3).

DISCUSSION

Infectivity of Friend leukemia in the 105,000 g. sediment of acellular fraction demonstrates that this disease can be transmitted to ddO mice by virus, as in the case of other strains of mice.

Unlike the polyoma virus, the Friend virus is apparently a strain specific virus. This virus can easily be transmitted to the adult Swiss mouse or DBA/2 mice, but not to the adult PRI, C5H, A, C57Bl/6 or to F1 (C58 x BALB) mice. Transfer of the Friend disease to dd strain mice has been reported to be successful in ddOM, ddD, and ddY. In this report, susceptibility of ddO mice was demonstrated. Occurrence of the Friend leukemia in ddO mice was indicated by proliferation of reticulum cells and erythroblasts in the spleen. Increased splenic radioiron uptake, progressive elevation of the hematocrit value and RBC count was also observed.
Inability to demonstrate erythropoietic activity in the plasma or the spleen extract from the infected mice by the polycythemic mouse bioassay method suggested that erythrocytosis in Friend leukemia was not induced by an increased production of endogenous erythropoietin; therefore, the speculation that erythroblastosis in Friend leukemia is caused by hemorrhage in the affected organs is not substantiated by our present studies. Moreover, the fact that transfusion-induced polycythemia did not suppress the erythropoiesis in Friend leukemia further supported our suggestion of the independence of this increased erythropoiesis from erythropoietin.

Two possible mechanisms for increased erythropoiesis in Friend leukemia are suggested: (1) the virus caused an increased mitosis of the erythroblasts, or (2) the virus caused the hematopoietic stem cell to differentiate into erythroblasts. In the latter case, the virus is thought to affect the stem cell in a similar way as erythropoietin.

Recent observations related to erythropoietin-induced stem cell differentiation indicated the intimate relationship between the changes in nucleic acid metabolism in the stem cell and erythropoietin action. These considerations could lead us to a tempting speculation that in Friend leukemia mice, the virus is substituted for erythropoietin, resulting in the increased and uncontrolled differentiation of stem cells to the degree of polycythemia.

SUMMARY

Friend leukemia infection in ddO mice produced a marked increase in the erythropoietic activity of the affected mice. By the fact that the increased erythropoietic activity was not suppressed by the transfusion polycythemia and that the plasma and spleen extract of the infected animal showed no erythropoietic activity, it is postulated that erythropoiesis in Friend leukemia is governed by mechanisms other than normal ones. The possible role of the virus in differentiating the stem cell into erythroblasts is discussed.

SUMARIO IN INTERLINGUA

Le infection de muses ddO con leucemia Friend produceva in le animales un marcate augmunto del activitate erythropoietic. A base del factos que le augmentate activitate erythropoietic non esseva supprimite per le polycythemia transfusional e que le plasma e le extracto splenic del inficite animales monstrava nulle activitate erythropoietic, il es postulate que erythropoiese in leucemia Friend es governate per mechanismos altere que le normales. Es commentate le rolo possibile del virus in le differentiation del cellula primordial in erythroblastos.

REFERENCES

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