Erythropoiesis in Friend Leukemia: Red Blood Cell Survival and Ferrokinetics

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Summary

RBC survival and ferrokinetics were evaluated in Swiss Webster female mice infected with Friend virus. Infection causes a hemolytic anemia associated with reticulocytosis and a compensatory increase in erythropoiesis in the spleen. The RBC survival studies were performed by noncohort label (51Cr) and cohort label (59Fe) technics. The anemia and shortened survival of RBC occurred in nonsplenectomized and splenectomized animals. Ferrokinetic studies did not reveal ineffective erythropoiesis. The data suggest a direct effect of the Friend virus on the RBC leading to a hemolytic anemia.

Introduction

The Friend virus causes a leukemia characterized by the early development of thrombocytopenia in the peripheral blood and by reticulocytosis in the spleen (6,8-11). Significant thrombocytopenia occurs 2 days after infection and is a major factor in the hemorrhagic disorder that results in the early death of affected animals (6). Approximately 3-4 days after infection, enlarging foci of reticulum cells surrounded by accumulations of erythroblasts in all stages of development are evident on histologic examination of the spleen (11). The relationship of these erythroid elements to the reticulum cells is not clear (11). Ludwig et al. (10) postulated that the altered erythropoiesis in Friend leukemia could be secondary to either hemolysis with a compensatory increase in erythropoiesis or the induction of an erythroleukemia by the Friend virus. On the basis of their pathologic studies in BALB/c and RF mice they suggested that the altered erythropoiesis was benign and not a form of erythroleukemia.

In the study reported herein, the survival of RBC from Friend-infected and normal mice was studied by means of noncohort labeling with radioactive chromium (51Cr) and cohort labeling with radioactive iron (59Fe). Studies were performed in both nonsplenectomized and splenectomized mice. Ferrokinetic studies, including the 59Fe t1/2, the plasma iron turnover, the reappearance of 59Fe in the RBC and the uptake of 59Fe in the spleen, were obtained in control and infected mice. The data in this study indicate that Friend virus infection in Swiss Webster female mice causes a hemolytic anemia associated with reticulocytosis and a compensatory increase in erythropoiesis in the spleen. On the basis of the reappearance of 59Fe in RBC there is no evidence for ineffective erythropoiesis.

Materials and Methods

Mice

Weanling female mice (16-18 gm) of the “general purpose” non-inbred Swiss Webster mouse strain from the Huntington Farms production colony (Philadelphia, Pennsylvania) were used in this work. They were maintained 10/cage, in plastic cages, and fed Purina laboratory chow with water ad libitum.

Mice were anesthetized with sodium pentobarbital (Nembutal) and then splenectomized through a left flank incision. The hilum of the spleen was ligated prior to removal of the spleen. This procedure was associated with only minimal blood loss. Splenectomized mice were permitted to recover for a minimum of 2 weeks before being used in experiments.

Virus

The Friend virus used in these experiments was obtained from Dr. Wallace P. Rowe, National Institute of Allergy and Infectious Disease, Bethesda, Maryland. This virus was maintained by the serial passage of whole blood at weekly intervals. Blood was obtained from infected animals by decapitation, 125 units of penicillin and 125 µg of streptomycin per ml of blood were added, and 0.2 ml immediately injected i.v. (tail vein). Normal control blood was collected and passed in a similar manner.

The virus was passed 11 times in our laboratory before the start of any experiment. The virus titer determined by the graded assay technic of Rowe and Brodsky was SED 2.1 (Spleen enlarging dose to 500 mg spleen weight) (15).

Red Cell Survival

1. NONCOHORT LABELING (51Cr). Six ml of whole blood were incubated at 37°C for 30 min with 200 μc of 51Cr (Chromotope, Squibb). After 30 min, 100 mg of ascorbic acid were added. The RBC survival of control and experimental groups was obtained by i.v. injection of 0.3 ml of 51Cr-labeled whole blood into the tail veins of normal nonsplenectomized mice. The infected whole blood was obtained 14 days after Friend virus infection from nonsplenectomized mice with spleens weighing at least 500 mg and infected splenectomized mice with packed cell volumes of 32% or...
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less. Starting on Day 1 after injection of \(^{51}\text{Cr}\)-labeled RBC, the mice were bled serially from the retroorbital venous plexus using 16 \(\mu\)l "double-oxalated" capillary tubes. The cpm of each sample were obtained and after the data were plotted on semilogarithmic paper, the % survival with time was determined. Using a formula for the elution of \(^{51}\text{Cr}\) from human RBC, the median RBC survival was calculated (7).

2. COHORT LABELING (\(^{59}\text{Fe}\)). Nonsplenectomized and splenectomized mice were given i.v. injections of 0.25 ml of \(^{59}\text{Fe}\) (Ferrutope, Squibb) containing 3 \(\mu\)c. Three hr later and daily thereafter they were given i.p. injections of 0.5 mg of nonradioactive iron [iron dextran injection, 50 mg elemental iron per ml (Imferon)] to block reutilization of \(^{59}\text{Fe}\) (2, 16). Individual mice were bled serially from the retroorbital venous plexus, and the cpm in 16 \(\mu\)l whole blood determined. The % survival with time was calculated from the peak reappearance of \(^{59}\text{Fe}\) and plotted on semilogarithmic paper. In the experimental groups, the mice were infected with Friend virus 7 days after the i.v. injection of \(^{59}\text{Fe}\). In this manner the RBC in control and experimental groups could be easily compared.

Routine Hematologic Parameters

Hemoglobin concentration, PCV,\(^3\) and reticulocyte count were performed by methods previously published (5).

Iron Determination

Serum iron was determined in individual mice by the microtechnic of Natelson (14). The normal serum iron in Swiss Webster female mice is 247 \(\pm\) 100 \(\mu\)g (5).

Ferrokinetics

Individual mice were given i.v. injections, in the tail vein, of 1.0 \(\mu\)c of \(^{59}\text{Fe}\) in a volume of 0.25 ml of physiologic saline solution. At 10, 30, and 60 min after injection, using a "double-oxalated" capillary tube, 16 \(\mu\)l of blood were obtained from the retroorbital venous plexus of each mouse. The radioactivity in the capillary tube was then determined in a well-type scintillation counter. The cpm in each specimen were plotted against time on semilogarithmic paper and the \(^{59}\text{Fe}\) t\(_1/2\) determined.

The PCV was determined in duplicate by the microhematocrit technic on blood obtained from the retroorbital venous plexus.

The plasma iron turnover rate was calculated by the method of Bothwell (3).

The \(^{59}\text{Fe}\) clearance curve was extrapolated back to zero time and the total counts at time zero (cpm\(_0\)) in 16 \(\mu\)l whole blood determined. The radioactivity of 16 \(\mu\)l of whole blood, obtained 24 hr after injection of \(^{59}\text{Fe}\), was divided by the cpm\(_0\) and % \(^{59}\text{Fe}\) reappearance in RBC calculated.

Organ Counting

The mice were exsanguinated via the retroorbital venous plexus and killed by cervical fracture. The uptake of \(^{59}\text{Fe}\) in the spleen and liver was determined 6 or 24 hr after injection of \(^{59}\text{Fe}\). The radioactivity in the individual livers and spleens was determined in a well-type scintillation counter. The % \(^{59}\text{Fe}\) uptake was obtained by dividing the cpm in either the liver or spleen by the cpm of a \(^{59}\text{Fe}\) standard prepared at the time of \(^{59}\text{Fe}\) injection. The % uptake was also expressed per gm of spleen or liver weight. Livers and spleens were weighed on a shadowgraph tumor weighing scale to the nearest 5 mg.

Results

RBC Survival

In Charts 1 and 2 are presented the data obtained with \(^{51}\text{Cr}\)-labeled RBC from normal and infected nonsplenectomized and splenectomized mice. The \(^{51}\text{Cr}\) t\(_1/2\) of normal RBC from nonsple-
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Days After CHART 3. Survival of cohort-labeled 69Fe RBC in normal non-splenectomized mice (X — X). Survival of cohort-labeled 59Fe RBC in nonsplenectomized mice infected with Friend virus 7 days after i.v. 59Fe (O — O).

CHART 4. Survival of cohort-labeled 69Fe RBC in normal spleenectomized mice (X — X). Survival of cohort-labeled 59Fe RBC in splenectomized mice infected with Friend virus 7 days after i.v. 59Fe (O — O).

nectomized mice is 12 days with a calculated median RBC survival of 23 days. RBC from infected nonsplenectomized mice have a 51Cr t½ of 7 days with a median RBC survival of 12 days. Therefore, in these experiments there is approximately a 50% decrease in RBC survival with Friend virus infection (Chart 1).

Similar data were obtained with RBC obtained from control spleenectomized and Friend-infected spleenectomized mice. (Chart 2). With control RBC the 51Cr t½ is 17 days with a median RBC survival of 38 days versus a 51Cr t½ of 5.5 days with a median survival of 9 days in the experimental group.

In Charts 1 and 2 each point represents the average of at least 10 control or infected mice.

In Charts 3 and 4 are presented the data obtained with 59Fe cohort labeling of RBC.

In Chart 3 are results obtained in control and infected nonsplenectomized mice. Each point on the chart represents the average of 15 mice. In the control group the RBC survival is 25 days, versus an RBC survival of 9 days in the infected mice.

As with 51Cr there is a sharp decrease in survival with infection. The increased RBC destruction begins within the first 48 hr after infection.

The data in splenectomized control and infected mice is similar (Chart 4). Control RBC survival is 27.5 days versus 9 days in the infected group. The data with 59Fe indicate a greater than 50% decrease in RBC survival with Friend infection.

A comparison of hemoglobin concentration, PCV, and retic 0, 7, and 14 days after infection with Friend virus in nonsplenectomized and splenectomized mice is presented in Table 1. Using a 2-way analysis of variance with correction for disproportionate numbers in subgroups, the data at 2 weeks indicate that in both groups there is a significant drop in Hb and PCV associated with a rise in retic count (P < 0.001). However, the decrease in Hb and PCV is significantly greater in splenectomized mice, and the reticuloctosis is less in the splenectomized group (P < 0.001).

Ferrokinetics

1. Reappearance of 59Fe in RBC at 24 hr; 59Fe t½; PIT. Ferrokinetic studies were performed 2, 4, 7, and 14 days after infection in nonsplenectomized mice. The data are presented in Table 2. Following infection there is a fall in PCV, but there is no definite change in 59Fe t½ and PIT as compared to normals. However, starting 4 days after infection, there is a consistently greater reappearance of 59Fe in the RBC at 24 hr as compared to control values.

In a control experiment a group of 22 normal mice were given injections of 0.2 ml of normal homologous RBC. There was no significant change in ferrokinetics by this procedure.

2. Uptake 59Fe in spleen and liver. The % uptake of injected 59Fe and the %/gm of spleen or liver weight is presented in Tables 2 and 3. Data for mice killed 24 hr after the injection of 59Fe are in Table 2, and the data for those animals killed 6 hr after 59Fe are in Table 3. There is a progressive decrease in the 24-hr % uptake of 59Fe/gm spleen with infection and at 14 days the uptake is below normal. However, the total 24-hr % uptake of 59Fe in the spleen is increased 7 and 14 days after infection. The increase in 59Fe % uptake parallels the increase in splenic size (Table 2).

The pattern of 59Fe uptake in the spleen 6 hr after i.v. 59Fe is similar to that obtained at 24 hr. The 24-hr % uptake is less than the 6-hr % uptake in control and infected animals. This is due to the release of iron from the spleen with the reappearance of more than 60% of the 59Fe in the peripheral blood by 24 hr.

Discussion

Infection with Friend virus in Swiss Webster female mice results in a hemolytic anemia as demonstrated by a shortened RBC survival, a fall in Hb, PCV, and reticuloctosis. The decreased RBC survival is evident with both noncohort (51Cr) and cohort labeling (59Fe). The results with 59Fe rule out the possibility that the decreased survival demonstrated with 51Cr might be related to a greater nonspecific elution of 51Cr from infected RBC as opposed to control RBC. The role of the spleen in the pathogenesis of this hemolytic anemia is also considered. One might suspect that the massive splenomegaly caused by the Friend virus would
### TABLE 1
**Hemoglobin Concentration, PCV, and Reticulocyte Count in Control and Infected Nonsplenectomized and Splenectomized Mice**

<table>
<thead>
<tr>
<th>Day after Infection</th>
<th>Nonsplenectomized</th>
<th>Splenectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb (gm %)</td>
<td>PCV (%)</td>
</tr>
<tr>
<td>0</td>
<td>12.9 ± 0.6 (10)^a</td>
<td>45.4 ± 1.7 (10)</td>
</tr>
<tr>
<td>7</td>
<td>11.5 ± 1.7 (16)</td>
<td>38.7 ± 4.0 (16)</td>
</tr>
<tr>
<td>14</td>
<td>10.6 ± 1.6 (15)</td>
<td>35.5 ± 4.5 (15)</td>
</tr>
</tbody>
</table>

* Abbreviations: PCV, packed cell volume; retic, reticulocyte count.

**Mean ± S.D. Numbers in parentheses represent number of mice.**

### TABLE 2
**Ferrokinetic Studies in Swiss Webster Female Mice after Infection with Friend Leukemia Virus, and in Normal Swiss Webster Female Mice 24 hr after Injection of \(^{59}\)Fe**

<table>
<thead>
<tr>
<th>Time after infection (days)</th>
<th>PCV^a (%)</th>
<th>(^{59})Fe T1 (min)</th>
<th>Plasma Fe (µg %)</th>
<th>PIT^b</th>
<th>(^{59})Fe spleen (%)</th>
<th>Spleen (gm)</th>
<th>(^{59})Fe spleen (%/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>37.4 ± 3.4 (16)^a</td>
<td>64.2 ± 17.0 (16)</td>
<td>286.3 ± 58.6 (16)</td>
<td>2.8 ± 1.2 (16)</td>
<td>68.4 ± 18.8 (16)</td>
<td>8.0 ± 3.3 (21)</td>
<td>0.238 ± 0.067 (21)</td>
</tr>
<tr>
<td>4</td>
<td>38.3 ± 4.4 (22)</td>
<td>70.6 ± 28.3 (22)</td>
<td>220.1 ± 64.6 (22)</td>
<td>1.9 ± 0.7 (22)</td>
<td>80.5 ± 26.2 (22)</td>
<td>2.8 ± 1.4 (22)</td>
<td>0.167 ± 0.078 (22)</td>
</tr>
<tr>
<td>7</td>
<td>33.7 ± 5.0 (22)</td>
<td>87.5 ± 35.3 (22)</td>
<td>288.7 ± 69.4 (22)</td>
<td>2.6 ± 1.4 (22)</td>
<td>83.7 ± 21.1 (22)</td>
<td>17.9 ± 10.7 (22)</td>
<td>0.963 ± 0.448 (22)</td>
</tr>
<tr>
<td>14</td>
<td>29.7 ± 6.4 (21)</td>
<td>104.6 ± 74.9 (21)</td>
<td>212.9 ± 69.3 (21)</td>
<td>1.9 ± 0.9 (21)</td>
<td>80.1 ± 20.4 (21)</td>
<td>23.8 ± 11.1 (21)</td>
<td>1.754 ± 0.862 (21)</td>
</tr>
<tr>
<td>Normals</td>
<td>43.4 ± 4.0 (69)</td>
<td>61.7 ± 17.0 (166)</td>
<td>247.4 ± 107.2 (55)</td>
<td>2.4 ± 1.3 (55)</td>
<td>62.4 ± 18.1 (90)</td>
<td>4.0 ± 2.4 (33)</td>
<td>0.122 ± 0.046 (83)</td>
</tr>
</tbody>
</table>

* Abbreviations: PCV, packed cell volume; PIT, plasma iron turnover.

^a mg/24 hr/100 ml whole blood.

^b Mean ± S.D. Numbers in parentheses represent number of mice.

### TABLE 3
**Ferrokinetic Studies in Swiss Webster Female Mice after Infection with Friend Leukemia Virus and in Normal Swiss Webster Female Mice 6 hr after Injection of \(^{59}\)Fe**

<table>
<thead>
<tr>
<th>Time after infection (days)</th>
<th>PCV^a (%)</th>
<th>(^{59})Fe T1 (min)</th>
<th>Plasma Fe (µg %)</th>
<th>PIT^b</th>
<th>(^{59})Fe spleen (%)</th>
<th>Spleen (gm)</th>
<th>(^{59})Fe spleen (%/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>37.1 ± 4.4 (21)^c</td>
<td>98.6 ± 60.1 (21)</td>
<td>175.6 ± 51.2 (18)</td>
<td>1.5 ± 0.7 (18)</td>
<td>1.9 ± 1.1 (21)</td>
<td>0.188 ± 0.070 (21)</td>
<td>10.1 ± 3.2 (21)</td>
</tr>
<tr>
<td>7</td>
<td>30.5 ± 3.3 (22)</td>
<td>75.0 ± 55.5 (22)</td>
<td>103.7 ± 55.0 (22)</td>
<td>1.8 ± 0.9 (22)</td>
<td>28.5 ± 5.4 (22)</td>
<td>0.919 ± 0.261 (22)</td>
<td>32.4 ± 6.8 (22)</td>
</tr>
<tr>
<td>14</td>
<td>28.9 ± 9.2 (20)</td>
<td>90.3 ± 40.9 (21)</td>
<td>220.6 ± 54.5 (20)</td>
<td>2.0 ± 1.0 (20)</td>
<td>32.1 ± 11.8 (21)</td>
<td>1.201 ± 0.484 (21)</td>
<td>25.6 ± 21.8 (21)</td>
</tr>
<tr>
<td>Normals</td>
<td>43.4 ± 4.0 (69)</td>
<td>61.7 ± 17.0 (166)</td>
<td>247.4 ± 107.2 (55)</td>
<td>2.4 ± 1.3 (55)</td>
<td>13.7 ± 5.7 (33)</td>
<td>0.122 ± 0.046 (83)</td>
<td>140.2 ± 61.3 (33)</td>
</tr>
</tbody>
</table>

* Abbreviations: PCV, packed cell volume; PIT, plasma iron turnover.

^b mg/24 hr/100 ml whole blood.

^c Mean ± S.D. Numbers in parentheses represent number of animals.
produce hypersplenism with increased sequestration and destruction of RBC resulting in a hemolytic anemia. However, the decreased RBC survival in splenectomized infected mice is similar to that obtained in non-splenectomized mice. This indicates that the splenomegaly is not a major factor in causing the shortened RBC survival. Furthermore, infected splenectomized mice were more anemic than infected non-splenectomized mice. These data also obviate the possibility that the fall in Hb and PCV was due to trapping of RBC in the massively enlarged spleens. The $^{59}$Fe data also indicate that increased hemolysis develops within 48 hr after infection at a time when splenic enlargement is minimal. It is possible that the reticuloysis is in part responsible for the shortened RBC survival (16). However, the design of $^{59}$Fe cohort experiment is such that RBC precursors are labeled when erythropoiesis is normal. Seven days later infection is induced, and an immediate decrease in RBC survival occurs. The results of these experiments imply that the hemolytic anemia is caused by a direct effect of the virus on the RBC.

No significant change is noted in the $^{59}$Fe $t_1$ and PIT in non-splenectomized mice but the $^{59}$Fe reappearance in RBC is increased 7 and 14 days after infection when reticuloysis also develops. Mirand et al. (13) noted a normal $^{59}$Fe reappearance in RBC at 24 hr in Ha/ICR Swiss and DBA/1 mice 7, 14, 21, and 28 days after infection with Friend virus.

Since the mouse spleen from the standpoint of erythropoiesis is analogous to bone marrow (5), the uptake of $^{59}$Fe in the spleen was determined 6 and 24 hr after the administration of i.v. $^{59}$Fe. Four days after infection the 6-hr $^{59}$Fe uptake/gm spleen is suppressed as compared to the normal controls. Subsequently the $^{59}$Fe uptake/gm spleen increases but is still below the normal range. However, the total % $^{59}$Fe uptake by the spleen increases with infection and at 7 and 14 days is above the normal range. This apparent disparity between the % uptake/gm spleen and the total % uptake is probably related in part to the great increase in splenic weight. The increased splenic weight in Friend leukemia is caused not only by erythroid hyperplasia but also by lymphocytic and reticulum cell hyperplasia (11). Since at 6 hr in the normal mouse, $^{59}$Fe is completely cleared from the plasma, the data indicate that total erythropoiesis in an enlarged Friend spleen is greater than normal. However, on a per gm basis, erythropoiesis is actually decreased as compared to normal.

The 24-hr % $^{59}$Fe uptake/gm spleen decreases with duration of infection while the total uptake of $^{59}$Fe increases and parallels the increase in splenic weight. These observations at 24 hr are similar to those reported by Mirand et al. (13). Although in our experiments, the results 6 and 24 hr after i.v. $^{59}$Fe are similar, as a rule, one should not wait 24 hr after i.v. $^{59}$Fe injection to assess erythropoiesis in the mouse spleen because at 24 hr over 60% of the $^{59}$Fe has reappeared in the peripheral blood (5). Therefore, at this time other variables such as iron storage, reutilization of $^{59}$Fe and RBC sequestration are measured.

The ferrokinetic data indicate that hemolysis with Friend virus infection is associated with an increased reappearance of $^{59}$Fe in RBC and a total increase in erythropoiesis in the enlarging spleen. Removal of the spleen increases the severity of the anemia and thereby indicates that the increased erythropoiesis in the spleen is in part a compensatory response to hemolysis. In the adult mouse the spleen is normally an active site of erythropoiesis and this type of a response to hemolysis might be anticipated (5).

There is no evidence for ineffective erythropoiesis, i.e., an accelerated $^{59}$Fe $t_1$ with a depressed $^{59}$Fe reappearance.

Because this work was performed in Swiss Webster mice, it is also important to consider the possibility of different erythropoietic responses in inbred strains of mice. Ludwig et al. (10) suggest that the hematologic response to Friend virus could depend on the titer of the virus preparation and the strain of mouse infected. They emphasize the importance of host factors such as genetic background and hormonal agents in determining the effect of tumor viruses, chemical carcinogens, and radiation. With Friend virus infection, Ludwig et al. (10) note differences in hematologic response between BALB/c and RF strains of mice. The BALB/c and Swiss Webster mice are both highly susceptible to Friend virus whereas RF mice are not. BALB/c mice surviving 12 or more days after infection have decreased numbers of erythroblasts in the marrow whereas the RF mice 16–80 days after infection demonstrate marrow erythroblastosis (10). Mirand et al. (12, 13) demonstrated a polycythemia in Friend-infected Ha/ICR Swiss and DBA/1 mice, but the polycythemia was not observed in the Ha/ICR Swiss mice after splenectomy. Although we have never observed a polycythemic response in Swiss Webster infected mice, our data also indicate that splenectomy modifies the response to Friend virus infection.

Ludwig et al. (10) on the basis of pathologic studies concluded that the altered erythropoiesis in Friend leukemia was consistent with the concept of erythroleukemia and not hemolysis. This conclusion was based on the observation that in infected mice histologic examination of the mesenchyme in liver, spleen, marrow, and lymphoreticular organs did not reveal pathologic or excessive hemosiderin deposits. In human erythroleukemia (Di Guglielmo), iron overload can generally be demonstrated and ferrokinetic studies reveal ineffective erythropoiesis (2). However, in certain human myeloproliferative disorders such as polycythemia vera and chronic myelogenous leukemia decreased iron stores in the marrow are observed early in the disease and there is no ineffective erythropoiesis (2, 4). Our studies in Swiss Webster female mice with Friend leukemia indicate that the altered erythropoiesis is primarily caused by a hemolytic state. One can only speculate as to whether this mechanism is similar to that noted in human chronic myelogenous leukemia (1), or whether the hemolysis is strictly a benign manifestation of infection with Friend virus.

References

6. Dennis, L. H., and Brodsky, I. Thrombocytopenia Induced...
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