Inhibition of Erythropoiesis by a Factor Present in the Plasma of Patients with Erythroblastopenia

By JOANNE H. JEPSON and LOUIS LOWENSTEIN

Pure red cell aplasia has been associated with a variety of conditions, including thymic tumors. It has recently been demonstrated that the thymus is involved in immunity in animals, and previous investigators have suggested that various erythroblastopenias are on the basis of autoimmunity although antibodies have not been demonstrated. The purpose of this paper is to report observations demonstrating inhibition of erythropoiesis in mice by a factor present in the plasma of two patients with erythroblastopenia. For brevity, only the case histories of these two patients will be presented.

CASE REPORTS

Case 1: Pure Red Cell Aplasia with Thymoma

An anterior mediastinal mass was first noted in this 56-year-old woman in 1948. Hematologic abnormalities were not observed until 1961, when the hemoglobin was found to be 8.9 Gm. per cent and no reticulocytes were present. The bone marrow showed complete absence of erythroblasts but granulopoiesis and thrombopoiesis were normal. Forty per cent of the cells were small lymphocytes, plasma cells and small unidentifiable cells with pyknotic nuclei. Aside from a persistent eosinophilia and an elevated A-2-globulin (1.85 Gm. per cent), hematologic studies were unremarkable. The patient showed no improvement following removal of a benign thymoma or after prolonged administration of large doses of prednisone and testosterone enanthate (400 mg./week). She received intermittent blood transfusions over the next 2 years, toward the end of which she required weekly transfusions and developed hemosiderosis. In 1963, following a left nephrostomy for hydronephrosis associated with impaired renal function, a mixed urinary tract infection proved refractory to antibiotic therapy. She developed a gram-negative septicemia and died in June, 1963.

Case 2: Acute Erythroblastopenia

A 32-year-old woman was admitted to hospital with seborrheic dermatitis for which she had received no known potentially toxic drug. On admission, her hemoglobin was 9.7 Gms. per cent; WBC was 3700; platelets were normal and reticulocytes were absent. Coombs' test and Heinz-body preparations were negative and serum bilirubin was normal. Bone
marrow aspirates revealed complete aplasia of erythroblasts, a slight plasmacytosis (8 per cent), and normal megakaryocytopoiesis and granulocytopoiesis except for an increase of eosinophil precursors. The serum gamma globulin was 2.29 Gm. per cent.

Figure 1 shows the course of her disease. Her hemoglobin decreased to 4.5 Gm. per cent over 1 month and she was transfused with 1400 ml. of blood. In view of the observed erythropoietic effect of prolactin* in the mouse,22 20 mg. of ovine prolactin was injected twice daily intramuscularly, from the 29th to the 37th hospital day, followed by 30 mg. twice daily for 2 more days (Fig. 1). An episode of cystitis responded to sulfisoxale but she developed persistent fever. After discontinuing the sulfisoxale the fever disappeared. On the 45th hospital day, oral prednisone therapy was started (40 mg. day); 36 hours later her reticulocytes rose to 2.2 per cent and reached a peak of 13 per cent 4 days later. Over the next two weeks the hemoglobin rose from 9.1 to 12.0 Gm. per cent. At this time her bone marrow revealed normoblastic hyperplasia and a normal number of lymphocytes and plasma cells. Prednisone was continued for a total of 6 weeks. One month after its discontinuation her hemoglobin was 13 Gm. per cent and the reticulocytes 2.6 per cent. She remained hematologically normal over the next 5 months without further therapy.

Case 3: Chronic Erythremic Myelosis (82-year-old white male)

Case 4: Idiopathic Hypoplastic Anemia (27-year-old female)

Case 5: Chloramphenicol-Induced Hypoplastic Anemia (35-year-old female)

METHODS AND EXPERIMENTAL PROCEDURE

Collection of Plasma and Urine

All blood was collected in heparinized syringes, immediately centrifuged and the plasma stored at −20 C. Urine was collected with 0.1 per cent phenol as preservative, kept at 5 C. during collection, and then at −20 C. until extraction. All urines were extracted by a modification23 of the procedure of Gordon.24 The final extract was stored at −5 C. For brevity, symbols are used in the figures for test materials as indicated in Table 1. The hemoglobin and state of the bone marrow at the time of urine and plasma collection are shown in Table 1.

Polycythemic Mouse Assay

Assay of the erythropoietic activity of test materials was performed in polycythemic mice as previously described,22 using the 48-hour Fe59 incorporation into erythrocytes as a measure of erythropoietic activity. The polycythemic mice received 25 mg. of urine extract and 0.5 to 1 ml. of plasma in the various combinations listed in Table 2.

Administration of Test Materials to Normal Mice and Mice Exposed to 24 Hours of Hypoxia

To determine the effect of injection of test plasmas on incorporation of Fe59 into erythrocytes of normal mice and of mice stimulated to produce endogenous erythropoietin (ESF), 50 male mice were divided into 5 equal groups. Intraperitoneal injections of 1 ml. volume of test materials were then made as follows: Group I received plasma from the patient with chloramphenicol-induced hypoplastic anemia; Group II received plasma from the patient with acute erythroblastopenia collected during the crisis; Group III received plasma from the same patient collected during remission; Group IV received normal human plasma; Group V received normal saline. Half of the mice from each group received intravenous injections of 0.5 με of Fe59 C13 at the same time as the plasma was injected. The rest of the mice from each group were exposed to a 10 per cent O2 environment for 24 hours25 to stimulate endogenous ESF production, and were injected intravenously with Fe59 C13

*Ovine prolactin was kindly supplied by Merck Research Institute, Westpoint, Pa., and Rahway, N. J., and made available by Dr. E. McGarry and Dr. J. C. Beck.
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Fig. 1.—Clinical course of acute erythroblastopenia in a 32-year-old woman treated with prolactin.

upon removal. All mice were exsanguinated 48 hours after the Fe59 C13 was injected, and the incorporation of Fe59 into erythrocytes was determined.

RESULTS

Assay of Test Materials in Polycythemic Mice

The effect of the test materials on the incorporation of Fe59 into erythrocytes of polycythemic mice are recorded in Figure 2 and Table 2. It is apparent that the erythropoietic activity of the urine extracts was inhibited by the simultaneous administration of plasma from the patient with acute erythroblastopenia, collected during both the crisis and the recovery phases. The effect of exogenous ESF was inhibited by as little as 0.5 ml. of plasma from the patient with red cell aplasia and thymoma. Although some inhibition was produced by normal human plasma, the effect was small (17 per cent) and was not comparable to that produced by the above plasmas. In contrast, plasma collected from the other patients did not have an inhibitory effect. While the plasma collected from the patient with the thymoma was erythropoietically active (21.7 per cent), her urine extract was inactive (1.81 per cent). Injection of her plasma and urine extract, simultaneously, inhibited the response to her plasma alone (4.89±.64). Incorporation of Fe59 into erythrocytes of mice receiving an extract of normal urine (0.32±.06), normal human plasma (1.39±.32) or saline (0.54±.05) was negligible.

Effect of Plasma on Erythropoiesis in Normal Mice and Mice Exposed to 24 Hours of Hypoxia

The effect of test materials on the incorporation of Fe59 into erythrocytes of these mice is recorded in Table 3 and Figure 3. It is apparent that both groups of mice receiving plasma collected from the patient with erythroblastopenia,
Table 1.—Level of Hemoglobin and State of Bone Marrow at the Time of Urine and Plasma Collection

<table>
<thead>
<tr>
<th>Donor</th>
<th>Hemoglobin (gm per cent) at time of collection</th>
<th>State of the bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (NHP)</td>
<td>15 (13-16)</td>
<td>normal</td>
</tr>
<tr>
<td>Red-cell aplasia with thymoma (ET)</td>
<td>6.0</td>
<td>Erythroblastopenia</td>
</tr>
<tr>
<td>Acute erythroblastopenia (AEP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>crisis</td>
<td>7.2</td>
<td>Erythroblastopenia</td>
</tr>
<tr>
<td>recovery</td>
<td>13.0</td>
<td>Normoblastic hyperplasia</td>
</tr>
<tr>
<td>Chronic erythremic myelosis (CEM)</td>
<td>11.0</td>
<td>Hyperplasia: 80% erythroid precursors</td>
</tr>
<tr>
<td>Idiopathic pancytopenia (CPC)</td>
<td>8.0</td>
<td>Panhypoplasia (megaloblastoid changes)</td>
</tr>
<tr>
<td>Chloramphenicol-induced hypoplastic anemia (CHA)</td>
<td>6.0</td>
<td>4.6 5.5</td>
</tr>
</tbody>
</table>

during both the crisis and recovery phases, showed significant inhibition of incorporation of Fe$^{59}$ into erythrocytes, whereas normal plasma had no effect and the plasma collected from the patient with chloramphenicol-induced hypoplastic anemia had only a very small inhibitory effect which fell within the sensitivity of the assay.

**DISCUSSION**

Both in vivo and in vitro neutralization of the ESF of various species by anti-ESF has been demonstrated previously, suggesting that an antibody reaction directed against ESF exists. Others have suggested the existence of antibodies or other inhibitors acting upon the bone marrow generally or acting specifically upon a single bone marrow cell type.

Plasma from the patient with thymoma and red cell aplasia inhibited the erythropoietic response of polycythemic mice to exogenous human ESF. This plasma was able to stimulate erythropoiesis in polycythemic mice although it had failed to stimulate erythropoiesis in the patient. These findings may be due to failure of a defective bone marrow to utilize normal ESF, or due to a molecular alteration of ESF occurring in the presence of an inhibitor, which makes it ineffective in the patient. The absence of erythropoietic activity in this patient's urine may have been due to (1) impaired excretion of erythropoietin in her urine, (2) inactivation of ESF by proteolytic enzymes, or (3) failure of our extraction procedures to isolate a chemically altered "erythropoietin." Reduction of the erythropoietic response produced by the plasma occurred when it was concurrently injected with her urine extract, suggesting that her urine contained an erythropoietic inhibitor which may be a specific
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antibody to ESF. Unfortunately, a complete study of this patient was interrupted by her death. Plasma from the patient with acute erythroblastopenia inhibited erythropoiesis in the normal mouse, the erythropoietic response of the mouse exposed to hypoxia and of the polycythemic mouse receiving injections of exogenous ESF. This inhibitory effect was similar to that produced by injection of antisera to ESF. The presence of an inhibitor in plasma collected in the immediate post-crisis period did not seem to impair the erythropoietic response of the patient. There is insufficient data to explain this phenomenon.

The small inhibitory effect observed in polycythemic mice receiving normal human plasma and exogenous ESF may be within the experimental error of the assay. However, it may indicate that a physiologic “inhibitor” is present, which only becomes apparent when tested in mice who are sensitive to erythropoietic factors, since the erythropoietic response of normal mice and mice exposed to hypoxia was not affected by the injection of normal plasma.

Both of the patients studied had elevated globulins, eosinophilia and bone marrows in which plasma cells and small lymphocytes were increased, all of which have been implicated in immune mechanisms. During remission of the acute erythroblastopenia, the bone marrow eosinophilia, lymphocytosis and plasmacytosis disappeared. These observations support the hypothesis that an immunological response had occurred.

It has been shown that erythropoiesis is stimulated by prolactin administration in the mouse, and it is known that some of its metabolic effects occur after the hormone has been discontinued. Following prolactin administra-
Table 2.—Effect of Combination of Plasma and Urine Extracts on the Incorporation of Fe** into Erythrocytes of Polycythemic Mice

<table>
<thead>
<tr>
<th>Plasma Source and Amount</th>
<th>Chloramphenicol Hypoplastic Anemia</th>
<th>Acute Erythroblastopenia</th>
<th>Red Cell Aplasia with Thymoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Fe** (± SE) % Inhibit</td>
<td>% Fe** (± SE) % Inhibit</td>
<td>% Fe** (± SE) % Inhibit</td>
</tr>
<tr>
<td>Saline</td>
<td>35.97 (± 1.96) 26.51 (± 3.24) 1.81 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1 ml)</td>
<td>(21)†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal human plasma</td>
<td>29.84 (± 2.16) 29.09 (± 2.24) 1.33 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1 ml.)</td>
<td>(9) (p&lt;.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute erythroblastopenia</td>
<td>20.07 (± 4.0) 17% (4) 44% (p&lt;.025)</td>
<td>13.88 (± 79) 85% (5) 48% (p&lt;.001)</td>
<td>4.89‡ (5) 78%§ (p&lt;.001)</td>
</tr>
<tr>
<td>crisis</td>
<td>(1 ml.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute erythroblastopenia</td>
<td>5.12 (± 0.79) 85% (5) 48% (p&lt;.001)</td>
<td>13.88 (± 1.38) 48% (p&lt;.001)</td>
<td></td>
</tr>
<tr>
<td>recovery</td>
<td>(1 ml.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red cell aplasia</td>
<td>24.54 (± 2.02) 33% (5) 78%§ (p&lt;.001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>with thymoma</td>
<td>(0.5 ml.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic erythemic myelosis</td>
<td>46.85 (± 1.21) (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>myelosis</td>
<td>(0.5 ml.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol-induced</td>
<td>46.85 (± 1.21) 53.42 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypoplastic anemia</td>
<td>(1 ml.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic hypoplastic anemia</td>
<td>39.33 (± 3.46) (5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Level of significance = 0.025. ‡Fe** incorporation after injection of 0.5 ml. of ET plasma alone = 21.7 per cent ±3.78. †Number of mice in each group enclosed in parentheses. §Inhibition of plasma erythropoietic activity by urine extract.
### Table 3. — Effect of Injection of Plasmas upon Incorporation of FE¹⁵ into Erythrocytes of Normal Mice and of Mice Exposed to 24 Hours of Hypoxia

<table>
<thead>
<tr>
<th></th>
<th>Normal Human Plasma</th>
<th>Acute Erythroblastopenia (Crisis)</th>
<th>Acute Erythroblastopenia (Recovery)</th>
<th>Chloramphenical-Induced Hypoplastic Anemia Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mice</td>
<td>% Fe⁵⁰ Incorporation</td>
<td>(± SE)</td>
<td>% Inhibit</td>
<td>% Inhibit</td>
</tr>
<tr>
<td></td>
<td>38.77</td>
<td>±1.33 (16)</td>
<td>18.03</td>
<td>19.82</td>
</tr>
<tr>
<td></td>
<td>36.96</td>
<td>±1.97 (6)</td>
<td>±.88</td>
<td>±1.38</td>
</tr>
<tr>
<td></td>
<td>% Inhibit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;.10</td>
<td></td>
<td>53%</td>
<td>49%</td>
</tr>
<tr>
<td></td>
<td>p†</td>
<td></td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mice exposed to 24 hours of hypoxia</td>
<td>% Fe⁵⁰ Incorporation</td>
<td>(± SE)</td>
<td>% Inhibit</td>
<td>% Inhibit</td>
</tr>
<tr>
<td></td>
<td>52.28</td>
<td>±1.87 (14)</td>
<td>22.44</td>
<td>29.40</td>
</tr>
<tr>
<td></td>
<td>50.55</td>
<td>±2.42 (5)</td>
<td>±.21</td>
<td>±2.2</td>
</tr>
<tr>
<td></td>
<td>% Inhibit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;.10</td>
<td></td>
<td>57%</td>
<td>44%</td>
</tr>
<tr>
<td></td>
<td>p†</td>
<td></td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*One ml. of plasma injected into each mouse.
†Level of significance = <.025.
‡Figures in parentheses represent number of mice in each experiment.
normal mice
CHA mice exposed to hypoxia
NFIP

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Fig. 3.—Per cent inhibition of 48 hour Fe\(^{59}\) incorporation into erythrocytes following administration of plasma to normal mice and to mice exposed to hypoxia. Forty-eight hour RBC Fe\(^{59}\) incorporation of saline injected mice = 0.0 per cent.


tion, the patient with acute erythroblastopenia recovered. Corticosteroid therapy probably did not cause her erythropoietic response, since 36 hours would seem an insufficient time for reticulocytes to have emerged from the completely suppressed marrow. Spontaneous recovery cannot be excluded, but the recovery following prolactin administration is sufficiently suggestive to merit further investigation.

SUMMARY

An erythropoietic inhibitor was found in the plasma of each of two patients with erythroblastopenia. Because these plasmas exhibited similar biological behavior to that of antisera to ESF, it is postulated that the inhibitory effect of these plasmas may be due to the formation of antibody directed against ESF.

SUMMARIO IN INTERLINGUA

Un inhibitor del erythropoiese esseva trovate in le plasma de cata-un de duo patientes con erythroblastopenia. A causa del facto que le duo plasmas manifestava un comportamento biologic simile de illo de antiseros anti factor de stimulation erythropoietic, le postulato es presentate que le efecto inhibitori del plasmas in le presente casos esseva possibilemente causate per le formation de anticorpore dirigite contra factor de stimulation erythropoietic.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Bernard Cooper and Dr. Nannie de Leeuw for their cooperation and assistance in allowing us to study their patients, to Dr. E. E. McGarry for her interest and advice concerning the prolactin studies, and to Mrs. Marietta Foldiak for her technical assistance.
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