Sex Hormones and the Regulation of Erythroid Spleen Colonies Development of Fetal Liver Origin

By Ilan Bleiberg and Gershon Perah

The development of erythroid colonies of fetal liver hematopoietic cell origin in adult irradiated polycythemic mice was studied. It was found that orchidectomy sharply reduced the number of erythroid colonies developed in the spleen of these polycythemic male recipients. Estrogen injection to the orchidectomized polycythemic recipient did not further decrease the number of erythroid colonies developed. It is concluded that the development of erythroid colonies of fetal liver origin in polycythemic male recipients is maintained mainly by testicular testosterone. The complete suppression of these colonies in female recipients does not seem to be a result of inhibition by estrogen.

ERYTHROPOIETIN HORMONE is accepted as a major factor in stimulating erythropoiesis in adult mammalian organisms where dynamic equilibrium in hemopoiesis is maintained. Several other factors have been reported to take part in the regulation of erythropoiesis. A humoral factor inhibiting the stimulating effect of erythropoietin was reported.1,2 The microenvironment of the hemopoietic stem cell was suggested to play an important role in directing the pathway of differentiation.3 Androgens such as testosterone are known as stimulators of erythropoiesis,4 and the stimulation was demonstrated to take place via erythropoietin.5 Estrogen, on the other hand, was reported to inhibit erythropoiesis.6 Jenkins et al.7 claimed that bone marrow cells treated with estrogen before injection into irradiated mice increased the number of erythroid colonies in the spleen; they suggested that, whereas estrogen inhibits erythropoiesis in the bone marrow, it stimulates spleenic erythropoiesis.

Much less is known about the role of the erythropoietin and other factors regulating erythropoiesis in the various stages of the developing embryo. Previous studies in this laboratory investigated the regulation of hematopoietic fetal liver cell differentiation in adult male spleen, based on the in vivo cloning method of Till and McCulloch8 for hemopoietic cell differentiation in mouse spleen. It was found that low levels of erythropoietin which did not stimulate erythropoiesis of adult bone marrow cells under polycythemic conditions did stimulate erythropoiesis of hematopoietic fetal liver cells.9 When fetal liver cells were injected into polycythemic male mice, erythropoietic activity took place under conditions where adult bone marrow cells underwent complete suppression. Such erythropoietic activity could not be demonstrated when the fetal liver cells were injected into female mice. Testosterone was found to evoke an erythropoietic stimulation of these cells in polycythemic female mice, an effect.
which was eliminated by antierythropoietin. There is insufficient evidence that testosterone alone is responsible for the erythropoietic activity of fetal liver cells in male mice. The present study is an attempt to elucidate the differences in expression of fetal liver cells in male and female recipients.

MATERIALS AND METHODS

Adult (C3HeB × C57BL) F1 male and female mice, 10–14 wk old, were exposed to 950 R total-body irradiation from a Cobalt-60 source (Gamma Beam 150 A, Atomic Energy of Canada Ltd.) at a focal skin distance of 75 cm and dose rate of 60 R/min. One hour after the irradiation, the animals were inoculated intravenously with $1.5 \times 10^5/0.5$ cc fetal liver cells. These cells were obtained from 12-day-old syngeneic fetuses, the suspensions prepared by passing the liver tissue through 25- and 27-syringe needles with Tyrode's solution. Half of the male recipients had been surgically orchidectomized about 40 days prior to irradiation. Polycythemia was induced by intraperitoneal injection of packed red blood cells from male and female donor mice on day 0 (1.0 ml) and day 1 (0.5 ml) postirradiation. To inhibit the colony-forming capacity of cells in transfused blood, whose blood was x-irradiated (1500 rads) prior to transfusion. The hematocrit level of the polycythemic hosts was checked before sacrifice and was found to be equal to or greater than 60%.

All experiments were terminated 6 days after inoculation with the fetal liver cell. The animals were killed by cervical dislocation, spleens were removed, fixed in Bouin's solution, sectioned, and stained in hematoxylin-eosin. Colonies were typed by testing five sections 100 μ apart from each spleen. Colonies containing a mixture of erythroid and granuloid cells were registered as erythroid colonies. Average number of erythroid colonies ± standard deviation is given. The results of t test statistical analysis is given in the text. Erythroid colonies are expressed per spleen. An area where more than 50 normoblasts are gathered is registered as erythroid colony.

Estradiol-17β (IkaPharm, Ramat-Gan, Israel), diluted in peanut oil to a concentration of 10 μg/ml, 0.1 ml containing 1 μg Estradiol, was injected subcutaneously to the appropriate mice.

RESULTS

The first question was whether removal of the testis as the main source of testosterone will abolish the erythropoietic activity of fetal liver cells in polycythemic male recipients. In the orchidectomized polycythemic male mice, injection with fetal liver cells resulted in a greatly reduced erythropoietic activity as compared with a similar but unorchidectomized group: the number of erythroid colonies on day 6 (Table 1) was 80% less in the orchidectomized group ($p < 0.01$). In the orchidectomized group in which polycythemia was not induced, only a small insignificant reduction (16%) in the number of erythroid colonies was observed in comparison with the unorchidectomized group ($p > 0.5$).

These results raise the question of the factor responsible for the stimulation of the erythroid colonies that did develop in the spleen of the orchidectomized

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Sex</th>
<th>Polycythemia</th>
<th>Orchidectomy</th>
<th>No. of Erythroid Colonies ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>M</td>
<td>–</td>
<td>–</td>
<td>8.3 ± 2.3</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>–</td>
<td>+</td>
<td>7.0 ± 1.9</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>+</td>
<td>–</td>
<td>5.4 ± 3.2</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>0.78 ± 1.59</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>–</td>
<td>–</td>
<td>9.2 ± 1.8</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>+</td>
<td>–</td>
<td>0</td>
</tr>
</tbody>
</table>

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Table 2. The Effect of Estradiol and Orchidectomy on Erythroid Colony Development of Fetal Liver Cell Origin

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Sex</th>
<th>Polycythemia</th>
<th>Orchidectomy</th>
<th>Estradiol</th>
<th>No. of Erythroid Colonies ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.06 ± 2.5</td>
</tr>
<tr>
<td>47</td>
<td>M</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>7.13 ± 2.83</td>
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<tr>
<td>36</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>7.1 ± 2.54</td>
</tr>
<tr>
<td>47</td>
<td>M</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>3.10 ± 2.9</td>
</tr>
<tr>
<td>47</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>0.42 ± 1.08</td>
</tr>
<tr>
<td>38</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.47 ± 1.05</td>
</tr>
<tr>
<td>49</td>
<td>M</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>2.38 ± 2.68</td>
</tr>
<tr>
<td>36</td>
<td>M</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>6.77 ± 2.25</td>
</tr>
<tr>
<td>52</td>
<td>F</td>
<td>-</td>
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<td>-</td>
<td>8.27 ± 2.86</td>
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<tr>
<td>48</td>
<td>F</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>0.08 ± 0.28</td>
</tr>
</tbody>
</table>

In a group of orchidectomized nonpolycythemic mice, adrenalectomy was performed to determine whether adrenal androgen steroids are responsible for this erythropoietic activity. In repeated experiments, these adrenalectomized mice could not bear the stress of irradiation and died before spleen colonies could be registered.

In the experiment (Table 2) on the suppressive effect of estradiol on erythropoietic activity of fetal liver cells in orchidectomized polycythemic mice, no additional reduction in the number of erythroid colonies was observed as a result of estradiol injection. Likewise, the effect of estradiol injection was studied in nonorchidectomized polycythemic male mice. An insignificant reduction was observed in this group as compared to a similar group not treated with estradiol ($p > 0.5$).

**DISCUSSION**

In previous work we demonstrated that bone marrow cells undergo complete erythropoietic suppression under polycythemic conditions, while the suppression of erythroid colonies of fetal liver origin in adult male mice spleen is only partial. The same fetal liver cells under polycythemic conditions in female mice undergo complete suppression. Testosterone injected into these female mice stimulated erythropoiesis in spite of the polycythemic condition. Anti-erythropoietin injected in addition to testosterone inhibited erythropoiesis. The conclusion was that stimulation of erythropoiesis of fetal liver cells in female mice by testosterone is via erythropoietin.

The fact that testosterone stimulates erythropoiesis of fetal liver cells in polycythemic female mice does not necessarily mean that this hormone is responsible for the erythropoietic activity in the male. Removal of testis, the main source of testosterone, resulted in a marked reduction in erythroid colonies. However, the fact that some erythroid colonies continued to be produced in these orchidectomized animals raised the possibility that adrenocortical androgens may be responsible for the erythropoietic activity. It was also postulated that the presence of estrogens in the female is responsible for the difference in the degree of suppression of fetal liver cell development between polycythemic orchidectomized mice and polycythemic female mice. These estrogen hormones are known to suppress erythropoiesis. Experiments
on the effect of androgens of adrenocortical origin in this erythropoietic activity failed, as the mice did not survive irradiation after adrenalectomy long enough to register spleen colonies. Medlinsky et al. reported that synthetic and antiandrogens neutralized erythropoietic activity of testosterone. It is our intention to test a similar substance to learn whether complete suppression of erythropoiesis will take place.

Estradiol injections in orchidectomized polycythemic male mice did not increase the degree of suppression of erythroid colonies development. Hence, estradiol is apparently not responsible for the difference observed in the degree of erythropoietic suppression between female and orchidectomized male recipients. Jenkins et al. reported stimulation of erythropoiesis in mice spleen after estradiol injection into nonpolycythemic mice and suggested that this stimulation may reflect a compensation for the suppressive effect of estradiol on bone marrow erythropoiesis. In the present work, we found no stimulation by estradiol. If any, a small insignificant reduction in incidence of erythroid colonies was observed in spleens of estradiol-injected nonpolycythemic orchidectomized mice, as compared to mice similarly treated but without estradiol injection ($p > 0.5$).

Kubanek et al. reported that complete suppression of erythroid colony development took place when fetal liver hemopoietic cells were injected into plethoric heavily irradiated recipients. However, these investigators used 16.5-day-old fetal liver cells, as compared to 12-day-olds used by us. Other differences in the experimental conditions, such as the extent of polycythemia and the strains of mice, might also be responsible for this discrepancy.

ACKNOWLEDGMENT
The authors wish to express their thanks to Professor Michael Feldman for his generous help throughout this work and to Mr. H. Otmy for his technical assistance.

REFERENCES
spleen colonies from fetal liver precursor cells.

Blood 42:185, 1973


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