The Factors Controlling Stem Cell Recirculation. II. ACTH-induced Inhibition of Migration of Hemopoietic Stem Cells

By G. I. Bezin, R. M. Khaitov, B. B. Moroz, R. V. Petrov, and O. O. Romashko

Injections of ACTH of prolonged action (twice, 1.5 units per mouse) given to lethally (800 R) irradiated mice with a hind limb shielded to the middle of the tibia brought about a twofold decrease in the number of spleen colonies. ACTH injections after sublethal whole-body irradiation (600 R) did not affect the number of endogenous spleen colonies. ACTH injection of normal mice brought about a substantial decrease in the CFU content in the circulating blood at the time of increased 11-hydroxycorticosteroid concentrations in the plasma. The results obtained are interpreted as inhibition of CFU migration from a shielded area of bone marrow induced by a high 11-hydroxycorticosteroid content in the plasma that followed ACTH injections which had no mitostatic effect on CFU proliferation.

The influence of bilateral adrenalectomy on the migration of hemopoietic stem cells from shielded areas of bone marrow in mice exposed to lethal x-irradiation was described previously. The elimination of adrenal function (hypocorticism) was found to increase the release of spleen colony-forming units (CFU) from bone marrow into the peripheral blood. It was presumed, therefore, that the migration of stem cells from bone marrow and their recirculation in the body were controlled by the hypophyseal-adrenal system.

The purpose of the present work was to study CFU migration from a shielded bone marrow area during lethal irradiation of the body with an increased corticosteroid level (hypercorticism) induced by ACTH injection.

MATERIALS AND METHODS

In the experiments (CBA x C57Bl) F1 mice 4-5 mo old weighing 23-28 g were used. The techniques of gamma irradiation and bone marrow shielding were described in detail previously. CFU migration from bone marrow was studied in lethally irradiated mice with a hind limb shielded to the middle of the tibia. To achieve the state of hypercorticism, long-acting ACTH-zinc phosphate was injected subcutaneously at a large dose (1.5 units per mouse, in 0.5 ml) immediately and 22 hr after irradiation. The ACTH solution prepared ex tempore was diluted with saline solution to adjust it to the required concentration. Control mice were injected with 0.5 ml of saline solution. In a series of experiments, mice after similar exposures were decapitated 2, 5, 8, 24, 26, 29, 32, and 48 hr after irradiation and the first ACTH injection. The level of 11-hydroxycorticosteroids (11 HOCS) was measured in the blood plasma by a modified fluorometric method using a Hitachi MPF-2A fluorescence spectrophotometer. The numerical data were treated statistically, and the arithmetic mean (M), the standard error (SE) and the 95% confidence interval or p value from the Student t test were calculated by the routine methods.

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Submitted July 5, 1974; accepted January 9, 1975.

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RESULTS AND DISCUSSION

As noted in Fig. 1, the content of 11-HOCS in the plasma increased to 82.9 μg/100 ml, i.e., tenfold (the level of 11-HOCS in normal mice was 8.3 μg/100 ml) 2 hr after the irradiation with a dose of 800 R and the first ACTH injection; it remained the same during the subsequent 6 hr and seemed to exceed the original value for a few hours more. The 11-HOCS concentration was somewhat lower than normal 24 hr after irradiation and ACTH injection (before the second injection). Similar changes in the 11-HOCS content were observed after the second injection of ACTH. However, the increase in the corticosteroid concentration was 2–2.5 times less than that after the first injection. The control mice showed an elevation in the 11-HOCS content to 30 μg/100 ml 2 hr after irradiation, but attained a subnormal level 5 hr postirradiation and afterwards.

Thus, after ACTH injections, CFU migration from bone marrow occurred under the hypercorticoid conditions. Table 1 shows that in control mice the number of CFU migrating from a shielded area of bone marrow to the spleen was 15.8 ± 1.4. ACTH injections resulted in a significant inhibition of CFU
migration: the number of CFU migrating from a similar area of bone marrow to the spleen was only 7.4 ± 0.9 (p < 0.001). Therefore, it may be concluded that an increase in the adrenal activity accompanied by a marked elevation of corticosteroids in the blood inhibited CFU migration. However, a decrease in the number of spleen colonies in ACTH-injected mice may result, not only from the inhibition of CFU migration, but also from the mitostatic effect of glucocorticoids. As is known, corticosteroids, and in particular hydrocortisone (at a dose of 25–50 mg/kg), inhibit CFU proliferation. This corticosteroid at smaller doses (5–10 mg/kg) does not produce such an effect.

Therefore, the ACTH effect on endogenous spleen colony formation in sublethally irradiated mice was studied. Mice were irradiated with a dose of 600 R and injected with ACTH (two times with an interval of 22 hr, 1.5 units per mouse). On the 10th postirradiation day, the control mice showed 6.7 ± 0.9 colonies formed due to proliferation of endogenous spleen CFU (Table 2). ACTH injection of the sublethally irradiated mice did not inhibit endogenous spleen colony formation. Thus, the degree of hypercorticism (Fig. 1) that developed in the irradiated mice after ACTH injections did not affect the proliferation of hemopoietic stem cells.

Cittadini et al. described a decrease in the number of endogenous spleen colonies in sublethally irradiated mice pretreated with ACTH (1 unit per mouse). However, unlike our procedures, these authors injected ACTH 8 hr before irradiation. Therefore, the decline in the number of endogenous spleen colonies in their experiments can be attributed to a lower influx of CFU from the bone marrow to the spleen due to ACTH-induced hypercorticism. This assumption is based on our earlier work which demonstrated that adrenalectomy (hypocorticism) resulted in a significant release of CFU from bone marrow into the peripheral blood and in their increased accumulation in the spleen of nonirradiated mice.

<table>
<thead>
<tr>
<th>Table 2. Effect of ACTH on the Endogenous Spleen Colony Formation in Sublethally (600 R) Irradiated Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation</td>
</tr>
<tr>
<td>Saline solution (control)</td>
</tr>
<tr>
<td>ACTH</td>
</tr>
</tbody>
</table>

*The data averaged from two experiments.*
Table 3. Effect of ACTH on the Circulating CFU in Nonirradiated Mice*  

<table>
<thead>
<tr>
<th>Preparation</th>
<th>2 hr</th>
<th>5 hr</th>
<th>8 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per 1 ml of blood</td>
<td>Per 10^6 of leukocytes</td>
<td>Per 1 ml of blood</td>
<td>Per 10^6 of leukocytes</td>
</tr>
<tr>
<td>Saline solution</td>
<td>27.5 ± 1.9(16)†</td>
<td>6.4 ± 0.4</td>
<td>19.0 ± 0.9(9)</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>ACTH</td>
<td>9.5 ± 4.9(13)</td>
<td>4.6 ± 2.4</td>
<td>2.5 ± 2.6(12)</td>
<td>1.1 ± 1.1</td>
</tr>
</tbody>
</table>

*The data averaged from two experiments.
†Injections were given at 9:00 a.m.
‡The number of mice is shown in parentheses.
This assumption was confirmed by additional experiments in which the CFU content in the peripheral blood was measured in nonirradiated mice after a single injection of ACTH with a dose of 1.5 unit per mouse. Table 3 shows that a noticeable decrease in the number of circulating CFU (as calculated per $1 \times 10^6$ leukocytes per milliliter of blood) occurred at the time of a high concentration of 11-HOCS in the plasma (2, 5, and 8 hr after ACTH injection). Twenty-four hr following the ACTH injection, when the 11-HOCS levels returned to normal, the CFU content reached the initial level.

Thus, it is very likely that a decrease in endogenous spleen colony formation as a result of preirradiation ACTH injections can be ascribed to the inhibition of CFU migration from bone marrow to the spleen before irradiation. Due to this, the sublethal irradiation occurs with the lowered content of endogenous CFU in the spleen. The lack of effects of ACTH injected after irradiation on the endogenous colony formation can be explained by the fact that CFU were not in the circulation of mice during the first week after irradiation with a dose of 600–700 R. It is likely that the action of ACTH on CFU migration is mediated through the release of endogenous corticosteroids from the adrenal cortex of the mouse, since similar results were obtained with the administration of exogenous hydrocortisone.

It is known that CFU circulation displays cyclic changes, e.g., the CFU concentration in the blood is twice as high in the morning as after midday. The corticosterone content in the plasma of mice is 2.6 times lower in the morning than after midday. These facts also give evidence that there is a close relationship between the corticosteroid level in the blood and the rate of recirculation of stem cells.

Thus, an intensive and prolonged stimulation of adrenal function leads to an inhibition of CFU migration from the bone marrow shielded during irradiation. The specific mechanism of this phenomenon requires further studies, since it remains unclear whether or not the inhibition of CFU migration to the blood flow can be accounted for by a lower permeability of the vascular wall and cell membranes induced by glucocorticoids. It is also important to clarify how the degree and duration of the hypercorticoid state affect the inhibition of CFU migration. These data are also needed to measure adequately the CFU migration rate in normal animals, because during the first postirradiation hours migration takes place under conditions of an increased corticosteroid level in the plasma (Fig. 1). In addition, it would be of interest to study the differentiation of stem cells whose migration has been altered.

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