CONCISE REPORT

Corticosteroid Effect on Murine Hemopoietic Precursor Cells In Vivo

By Eero Niskanen and John Squires

The effect of methylprednisolone on murine hemopoietic colony formation in diffusion chambers implanted in mice was evaluated. A dose-dependent increase in granulocytic colony (CFU-DG) formation from murine marrow was observed. This effect could be abrogated by administration of progesterone. These studies suggest that the murine early granulocytic precursors (CFU-DG) have receptors that mediate proliferation-promoting signals triggered by glucocorticoids. Erythroid colony formation (CFU-DE) was not affected by methylprednisolone administration.

R EPORTS of the effect of glucocorticosteroids on granulopoiesis have been contradictory. It has been demonstrated that administration of these hormones results in reduction of total CFU-C per femur and a decrease in serum colony-stimulating activity (CSA).1 Similar inhibitory effect has been noted in vitro when glucocorticosteroids have been added directly to tissue culture plates.1,4 On the other hand, enhanced regeneration of granulopoiesis following cyclophosphamide administration has been shown when the experimental animals were pretreated with methylprednisolone.5

In previous studies, the effect of corticosteroids on granulopoietic precursors, more primitive than CFU-C, has not been investigated. According to recent reports,6,7 the cells that form colonies in diffusion chambers in mice (CFU-DG) give rise to the cells that form colonies in vitro (CFU-C). In a further attempt to understand regulation of granulopoiesis by corticosteroids we studied the effect of methylprednisolone on CFU-DG formation.

MATERIALS AND METHODS

Cells

Female Swiss-Webster mice (Flow Laboratories, Inc., Rockville, Md.) weighing 24–32 g were used as marrow donors and diffusion chamber recipients. Contents of femora from two mice were suspended in McCoy’s 5A medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 20% heat-inactivated fetal calf serum (Flow Laboratories, Inc., Rockville, Md.), penicillin 100 U/ml, and streptomycin 50 μg/ml.

CFU-DG Assay

Diffusion chambers were prepared by glueing Millipore filters with a pore size of 0.22 μm onto both sides of a lucite ring and were sterilized under u.v. light for 2–4 hr. Each chamber was filled with 10⁶ mouse cells (0.1 ml) and 0.02 ml of citrated bovine plasma. Two diffusion chambers were implanted into the peritoneal cavity of each mouse. Methylprednisolone and progesterone were administered by daily intraperitoneal injections. Control animals were injected with the diluent without the hormone. On day 4 the chambers were removed from the mice. The clot attached to one filter was fixed for 6 min with phosphate-buffered 5% glutaraldehyde (pH 7.2) and washed with distilled water for 8 min. The preparation was then stained with benzidine-hematoxylin, dried, and placed in transparency medium 1.506 (R.P. Cargille Laboratories, Inc., Cedar Grove, N.J.). The clot was then examined at x 100 magnification. Groups of more than 19 granuloid cells were counted as colonies. Aggregates of 8 or more normoblasts were counted as erythroid colonies.

Statistical Method

Statistical analysis of results was performed using the t test.

RESULTS

Figure 1 shows that methylprednisolone increased CFU-DG formation in the diffusion chambers in vivo in a dose-dependent fashion up to a dose of 7.5 mg/kg. Further increase resulted in less stimulation. On the average, 4 erythroid colonies (CFU-DE) were detected in the control group. Administration of methylprednisolone had no effect on CFU-DE numbers.

Progesterone is known to block glucocorticoid binding to receptors and interfere with the transport of the receptor complex to the nucleus.8 Therefore, we injected mice simultaneously with progesterone and methylprednisolone. Progesterone (5 mg/kg daily) abrogated the statistically significant (p < 0.001) stimulatory effect achieved by methylprednisolone (5 mg/kg daily) administration. Progesterone per se had no effect on CFU-DG formation (Table 1).

DISCUSSION

In the present study we demonstrated that methylprednisolone stimulates granulocytic colony formation in diffusion chambers in mice in a dose-dependent fashion. This is in agreement with an earlier study suggesting that corticosteroids enhance granulopoiesis...
CORTICOSTEROIDS AND HEMOPOIESIS

2. Golde DW, Bersch N, Quan SG, Cline MJ: Inhibition of murine granulopoiesis in vitro by dexamethasone. Am J Hematol 1:369, 1976

Fig. 1. Effect of methylprednisolone on CFU-DG formation (± SE). Results from 3 replicate experiments.

The inhibitory effect on CFU-C observed both in vitro and in vivo can be explained on the basis of difference in responsiveness of granulopoietic precursors at different levels of maturation. Enhancement of granulopoietic recovery as judged by increased marrow CFU-C content on day 4 after concomitant cyclophosphamide and methylprednisolone administration may reflect a stimulatory effect on CFU-DG that overcomes the inhibition exerted on its progeny as judged by reduced marrow CFU-C content on day 1 following methylprednisolone administration. Another explanation is that the CFU-DG assay may be a better probe than the CFU-C assay of granulopoietic proliferative capacity in the bone marrow.

The stimulatory effect of methylprednisolone on CFU-DG was eliminated by simultaneous administration of progesterone. Analogously, the inhibitory effect on CFU-C can be abrogated with the same hormone. This suggests that the opposite effects of glucocorticoids on CFU-DG and CFU-C can be blocked by progesterone and that these effects are probably mediated via a specific receptor present on hemopoietic precursor cells or on accessory cells that modulate precursor cell proliferation.

In summary, our studies indicate that corticosteroids exert a stimulatory effect on precursor cells representing the earliest stage of granulopoietic differentiation.

ACKNOWLEDGMENT

We express our thanks to Gerda Pirsch and Glenda Pou for technical assistance, to Pat Harlow for typing the manuscript, to Dr. Peter Quesenberry for criticism, and to the Upjohn Company of Kalamazoo, Michigan for providing the methylprednisolone used in this study.

REFERENCES

<p>| Table 1. Effect of Corticosteroids on CFU-DG Formation |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>CFU-DG/Chamber ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>104 ± 10</td>
</tr>
<tr>
<td>Methylprednisolone*</td>
<td>168 ± 15</td>
</tr>
<tr>
<td>Progesterone*</td>
<td>94 ± 18</td>
</tr>
<tr>
<td>Progesterone* + methylprednisolone*</td>
<td>92 ± 12</td>
</tr>
</tbody>
</table>

Results from 3 experiments.

*5 mg/kg.
Corticosteroid effect on murine hemopoietic precursor cells in vivo

E Niskanen and J Squires