Effect of Adrenergic Agents on Hematopoiesis After Syngeneic Bone Marrow Transplantation in Mice

By Georges J.M. Maestroni, Ario Conti, and Ennio Pedrinis

We show that adrenergic agents modulate hematopoietic reconstitution after syngeneic bone marrow transplantation (BMT) in mice. Chemical sympathectomy by 6-hydroxydopamine (6-OHDA) significantly increased the number of peripheral blood leukocytes after syngeneic BMT. The α₁-adrenergic antagonist prazosin mimicked and extended the effect of 6-OHDA, with an additional rapid and significant increase of platelets, marrow granulocyte-macrophage colony-forming units, and nucleated spleen cells. Differential leukocyte counts and spleen histology from prazosin-treated mice confirmed that myelopoiesis was greatly enhanced and accelerated. In contrast, the β-adrenergic blocker propranolol abolished the prazosin-induced increase of platelets. The detailed mechanisms by which prazosin exerts these interesting effects remain to be elucidated.

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in humidified air and then examined by phase microscopy. Colonies containing more than 50 cells were counted as GM-CFU.

Statistics. Differences were analyzed by analysis of variance (ANOVA).

RESULTS

Effect of constant environmental lighting and chemical sympathectomy. Groups of mice were sympathectomized by one IP injection of 6-OHDA on day −2 or administered PBS as control. On day 0, the mice were lethally irradiated and, on day 1, transplanted with syngeneic BM and exposed either to L24 or to L12.

Figure 1 shows the effect of permanent lighting and/or of 6-OHDA on the number of PBL at various times after syngeneic BMT. Blood leukocytes were significantly lower in mice exposed to L24 than in control mice (L12, Fig 1). However, chemical sympathectomy by 6-OHDA abolished the effect of light and increased the leukocyte concentration even under normal conditions (L12). Such an increase was mainly due to granulocytes (data not shown).

Effect of adrenergic antagonists. We continued our studies using mice kept under a normal L12 photoperiodic cycle throughout the experiments. We treated groups of mice with the α1-adrenergic antagonist prazosin and/or with the β-adrenergic blocker propranolol after syngeneic BMT. We found that the daily SC administration of prazosin for 14 days induced a dramatic increase of the number of PBL, platelets, and spleen cells. As shown in Fig 2, the effect of prazosin was significant at doses ≥5 mg/kg BW. In a second set of experiments, the effect of prazosin adminis-
tered daily at the most effective dose of 10 mg/kg BW on leukocyte and platelet counts was evaluated after BMT. Figure 3 shows the results of such experiments and indicates that prazosin treatment started to show an effect on day 14. Differential leukocyte counts showed that the prazosin-induced increase was mainly due to increased granulocytes and monocytes (Fig 3). The number of GM-CFU in the transplanted marrow was consistent with such an effect and indicated that prazosin really enhanced granulocyte and macrophage reconstitution (Table 1). The number of nucleated spleen cells was also significantly increased by prazosin treatment, starting on day 14 (Fig 4). Consistent with the peripheral blood counts, histologic analysis of spleen sections from such mice showed a clear granulocytic hyperplasia (Fig 5).

Blockade of β-adrenergic receptors with propranolol was without influence in our model. However, when propranolol (10 mg/kg BW) was administered together with prazosin, it abolished completely the prazosin-induced increase of blood platelets (Table 2).

**DISCUSSION**

These results suggest that hematopoiesis is under an adrenergic regulation, at least during the regeneration phase after BMT. Both chemical sympathectomy and blockade of α₁-adrenergic receptors produced similar effects. In addition, BMT and the pharmacologic treatments apparently did not produce toxic effects nor infectious events that could account for the observed changes in hematopoiesis. In all the BMT experiments, the mortality rate was less than 2%.

Granulocyte and macrophage production may be under an inhibitory adrenergic tone, mediated by α₁-adrenoceptors, while platelet production may involve β-receptors. Apart from the well-known action of catecholamines on platelets functions and the reported sympatho-adrenal

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**Table 1. Effect of Prazosin Treatment on BM GM-CFU After Syngeneic BMT in Mice**

<table>
<thead>
<tr>
<th>Days</th>
<th>n</th>
<th>GM-CFU/Femur</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PRA</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>546 ± 137</td>
</tr>
<tr>
<td>14</td>
<td>12</td>
<td>1,572 ± 324</td>
</tr>
<tr>
<td>21</td>
<td>12</td>
<td>1,362 ± 313</td>
</tr>
</tbody>
</table>

The mean number of GM-CFU ± the standard deviation in the BM of C57BL/6 female mice that were treated with prazosin (PRA; 10 mg/kg BW) or saline (PBS) after syngeneic BMT. The number (n) of mice was obtained by sampling the animals from three different experiments.

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![Fig 3](image-url) Enhancing effect of prazosin on blood granulocytes and platelets after syngeneic BMT in mice. The mice were injected daily with (△) prazosin (PRA; 10 mg/kg BW) or with (○) PBS. Mean values ± standard deviation are relative to four experiments. The cumulative number of animals per group and point was 35 mice at day 7, 21 mice at day 14, and 16 mice at day 21. Such differences were due to the timed sampling of animals to killing for spleen cell count and GM-CFU assay. The horizontal bars represent the range of leukocyte and platelet concentrations in normal mice. GM/L, granulocytes-monocytes/lymphocytes ratio. a, P < .005; b, P < .002; c, P < .001.

![Fig 4](image-url) Effect of prazosin on spleen cell number after syngeneic BMT in mice. The number of spleen cells was evaluated in those mice whose blood platelet and leukocyte concentrations were reported in Fig. 3. The columns represent the mean value ± the standard deviation. a, P < .01.
influence on the immune response, we are not aware of any previous report describing an in vivo adrenergic modulation of hematopoiesis. 6-OHDA is known to induce a profound, although temporary, norepinephrine depletion. Its stimulatory effect on PBL was evident 7 days after BMT (Fig 1). Prazosin enhanced platelet production and spleen cells in addition to leukocytes, reaching significance on day 14 (Fig 2 through 5). The effect of prazosin on the number of GM-CFU per femur shown in Table 1 depended on a real increase of GM-CFU and not on an increased marrow cellularity, which was similar in all groups (data not shown).

This finding suggests that prazosin exerted its effect on the proliferation and differentiation of GM-progenitor cells after engraftment. The histologic appearance of the spleen confirmed that prazosin had an enhancing effect on myelo- poiesis. In regards to the reconstitution of platelets, the difference between chemical sympathectomy, which did not show any influence (data not shown), and the \( \alpha_1 \)-adrenergic blockade by prazosin (Figs 2 and 3) is explained by the effect of propranolol (Table 2). A minor point concerns the delayed effect of prazosin in comparison with that of 6-OHDA (Figs 1 and 3). A possible explanation might lie in

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**Fig 5.** Histology of spleen from prazosin (10 mg/kg BW) and PBS-treated mice, 14 days after syngeneic BMT. (A) Prazosin; (B) PBS. Hematoxylin-eosin stain was used. Original magnification \( \times 400 \).
the different speed and mechanism of adrenergic blockade attained by the two substances.13,14

Further in vivo and in vitro experiments are clearly needed to gain mechanistic insights into our interesting observations. However, taken together, our results suggest the possibility that an adrenergic regulation of hematopoiesis is operative also in normal physiologic situations. This would open up new perspectives in the study of lymphohematopoietic disorders ranging from idiopathic aplastic anemias and leukemias to immunodeficiencies. In any case, if the evidence of adrenergic regulation of BM reconstitution proves valid in humans, the implication in clinical BMT is amply evident. BMT is generally preceded by irradiation or chemotherapy that produces marrow aplasia and resultant pancytopenia. During the course of cytopenias, patients are at risk for opportunistic infections and/or hemorrhage.5,6 Pharmacologic adrenergic acceleration of hematopoietic reconstitution might mitigate the posttransplantation cytopenias and reduce the need for supportive care and/or treatment with hematopoietic growth factors.15

REFERENCES


TABLE 2. Effect of Propranolol on Blood Leukocyte and Platelet Concentration After Syngeneic BMT in Mice

<table>
<thead>
<tr>
<th></th>
<th>7 d L/μL</th>
<th>14 d L/μL</th>
<th>7 d P/μL</th>
<th>14 d P/μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (x10⁶)</td>
<td>(x10⁶)</td>
<td>(x10⁶)</td>
<td>(x10⁶)</td>
<td>(x10⁶)</td>
</tr>
<tr>
<td>PRA</td>
<td>6 0.95 ± 0.36</td>
<td>8.65 ± 1.93*</td>
<td>41 ± 6.3t</td>
<td>151 ± 23*</td>
</tr>
<tr>
<td>PRO</td>
<td>6 0.87 ± 0.24</td>
<td>4.06 ± 0.86</td>
<td>27 ± 3.7</td>
<td>98 ± 21</td>
</tr>
<tr>
<td>PRA + PRO</td>
<td>6 0.78 ± 0.22</td>
<td>7.91 ± 1.84*</td>
<td>22 ± 7.6</td>
<td>89 ± 29</td>
</tr>
<tr>
<td>PBS</td>
<td>6 0.82 ± 0.24</td>
<td>3.65 ± 0.57</td>
<td>26 ± 6.0</td>
<td>88 ± 14</td>
</tr>
</tbody>
</table>

Four-month-old C57BL/6 inbred mice were transplanted with syngeneic marrow as described. The mice were then injected SC with prazosin (PRA; 10 mg/kg), propranolol (PRO; 10 mg/kg), PRA + PRO, or PBS once a day for 14 days. The values are from one representative experiment and represent the mean ± the standard deviation of blood leukocyte (L) and platelet (P) counts performed 7 and 14 days after lethal irradiation and syngeneic BMT in C57BL/6 mice.

*P < .01
†P < .02
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