Impaired Erythropoietin Production in Mice Treated With Cyclosporin A

By Alessandro M. Vannucchi, Alberto Grossi, Alberto Bosi, Daniela Rafanelli, Stefano Guidi, Riccardo Saccardi, Renato Alterini, and Pierluigi Rossi Ferrini

Because recent data indicate that erythropoietin (Epo) production is defective in allogeneic bone marrow transplant (BMT) patients, we investigated the role of the immunosuppressive, nephrotoxic, agent cyclosporin A (CsA) on renal Epo production using an animal model. Mice were injected with 1.0 to 40.0 mg/kg/d CsA for 15 days. Thereafter, circulating Epo levels were evaluated in both intact animals and in mice made anemic with phenylhydrazine (PHZ). Serum Epo levels measured in CsA-treated animals were then compared with the predicted levels, which had been calculated in a reference population of normal, either intact or anemic, mice. In CsA-treated, intact animals both hematocrit and serum Epo levels were not significantly different from controls. However, serum Epo levels in CsA-treated, anemic mice were significantly lower than those expected in a control population of untreated, anemic mice with similar degrees of anemia. No significant increase in serum creatinine was recorded even at the highest doses of CsA used, nor were we able to document signs of renal toxicity by histologic examination of the kidneys. Therefore, therapeutical doses of CsA appear to affect the production of Epo under conditions in which the demand of the hormone is increased, as in response to anemia. We suggest that a subclinical kidney toxicity produced by CsA might have a role in the pathogenesis of the impaired Epo production observed in BMT patients, and may contribute to a delayed erythroid engraftment in at least some BMT patients.

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We were then compared to test if the difference between them was statistically significant. Moreover, we defined as "inappropriately low" for any given Hct value those serum Epo levels that were below the lower 95% confidence limit of the curve drawn for normal mice.

In normal animals, either intact or anemic (Hct ranging from 22% to 45%), serum Epo (U/L) and Hct (%) were inversely related (r = -0.93; P < .001) and the regression was described by the curve: y = -0.205x + 12.8, where y = Ln (serum Epo) and x = Hct. The parameters of this curve have been used to calculate, for any Hct value recorded in both controls and CsA-treated mice, the predicted serum Epo levels. The actually measured and the predicted values were then compared to test if the difference between them was statistically significant. Moreover, we defined as "inappropriately low" for any given Hct value those serum Epo levels that were below the lower 95% confidence limit of the curve drawn for normal mice.

In mice injected with 1.0 to 40.0 mg/kg/d CsA, serum CsA levels ranged from 46.0 to 630.0 ng/mL (Table 2). No significant increase in serum creatinine levels was observed, even in mice receiving the highest doses of CsA (Table 2). Histologic findings of the kidneys from CsA-treated animals were similar to those of control and normal mice (not shown in detail). In particular, we were unable to detect signs of tubular or vascular interstitial involvement, which are typical of CsA-induced renal toxicity.15

Hct and serum Epo levels measured in intact mice that had been treated with CsA (1.0 to 40.0 mg/kg/d) were not significantly different from controls (Table 2). However, serum Epo levels measured in mice receiving CsA in doses ranging from 10.0 to 40.0 mg/kg/d and made anemic with PHZ were significantly lower than the predicted values (Fig 1). A detailed analysis of experimental points showed that 39 of 45 serum Epo values (87%) measured in CsA-treated, anemic mice were inappropriately low when compared with normal mice with similar Hct values. No evident correlation between CsA dosage and serum Epo levels was observed; in fact, the amounts of serum Epo measured in mice receiving 10.0, 20.0, and 40.0 mg/kg/d CsA were, respectively, 55%, 64%, and 43% of the predicted value, and the difference among these groups of mice was not statistically significant. Control mice receiving vehicle oil only, either intact (Table 2) or anemic (not shown), did not behave differently from normal mice.

The lower than predicted serum Epo levels in CsA-treated mice were not just the results of a left shift of the regression line between serum Epo and Hct. In fact, as shown in Fig 2, the parameters of the curve describing the regression between serum Epo and Hct in mice with Hct ≤63% were y = -0.175x + 12.0 for normal animals, and y = -0.082x + 8.24 for CsA-treated mice. Both the slope and intercept values of these two lines were significantly different (P < .01 and P < .05, respectively).

**RESULTS**

In normal animals, either intact or anemic, circulating levels of the hormone Epo are finely regulated by renal Epo-producing cells in response to hypoxic stimuli, and a strict relationship normally exists between Hct and serum Epo levels. Beyond patients with severe renal diseases, in whom serum Epo levels are very low and not correlated with Hct values, some recent reports indicate that a defective Epo production may be also found in other pathologic conditions.14,15 One particular situation is represented by the patients undergoing BMT, because in several studies an inappropriate Epo production in response to anemia was found to be a characteristic of BMT, but not of ABMT.12 To elucidate the pathogenetic mechanism(s) responsible for this intriguing difference, we have focused our attention on the relationship between CsA dosage and serum Epo levels.

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**DISCUSSION**

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**Table 1. Treatment Schedule of the Six Groups of Mice Studied**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Anemia induction</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>No</td>
<td>Normal, intact mice</td>
</tr>
<tr>
<td>No treatment</td>
<td>Yes</td>
<td>Normal, anemic mice</td>
</tr>
<tr>
<td>Vehicle oil</td>
<td>No</td>
<td>Control, intact mice</td>
</tr>
<tr>
<td>Vehicle oil</td>
<td>Yes</td>
<td>Control, anemic mice</td>
</tr>
<tr>
<td>CsA</td>
<td>No</td>
<td>CsA-treated, intact mice</td>
</tr>
<tr>
<td>CsA</td>
<td>Yes</td>
<td>CsA-treated, anemic mice</td>
</tr>
</tbody>
</table>

Details of the dosage and timing of CsA treatment are provided in Materials and Methods, as well as details about the induction of anemia with PHZ.

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**Table 2. Serum CsA, Creatinine, and Epo Levels and Hct Values in Normal, Control, and CsA-Treated Intact Mice**

<table>
<thead>
<tr>
<th>Serum CsA (mg/kg/d)</th>
<th>Serum Creatinine (g/dL)</th>
<th>Hct (%)</th>
<th>Serum Epo (mU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mice (n = 10)</td>
<td>—</td>
<td>0.4 ± 0.2</td>
<td>42.7 ± 2.7</td>
</tr>
<tr>
<td>CsA-treated mice (mg/kg/d)</td>
<td>1.0 (n = 10)</td>
<td>46 ± 15</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>10.0 (n = 20)</td>
<td>210 ± 46</td>
<td>0.4 ± 0.1</td>
<td>42.0 ± 1.5</td>
</tr>
<tr>
<td>20.0 (n = 20)</td>
<td>360 ± 70</td>
<td>0.5 ± 0.1</td>
<td>41.4 ± 2.0</td>
</tr>
<tr>
<td>40.0 (n = 20)</td>
<td>630 ± 67</td>
<td>0.4 ± 0.1</td>
<td>42.5 ± 2.2</td>
</tr>
</tbody>
</table>

CsA-treated mice were injected IP with the indicated amount of CsA once a day for 15 days; control animals received vehicle oil only. Serum CsA and creatinine levels were assayed in pooled serum samples from two to three mice, and at least five pools were examined for each experimental group. Shown is the mean ± SD of data derived from four different experiments. Serum Epo levels were measured in individual samples. The number (n) of mice examined in a total of four different experiments is shown.
possible role of CsA, which is used for the prevention of graft-versus-host disease (GVHD) in BMT and is nephrotoxic at doses very close to the therapeutical ones.

In this regard, it is of interest the study of Abedi et al., who observed that BMT patients receiving methotrexate plus CsA for the prevention of GVHD had significantly lower levels of serum Epo than patients transplanted with a T-cell-depleted BM graft who did not receive any immunosuppressive therapy. Similarly, Besarab et al. found that the increment in serum Epo levels after renal transplantation occurred earlier in non-CsA-treated patients than in those receiving CsA. This finding led these investigators to speculate that CsA had some inhibitory effects on Epo production in the kidney. However, the toxic effects appeared to be different from those causing the tubular and vascular damage, because serum Epo levels were similarly low in CsA-treated patients, irrespective of whether they had increased creatinine levels.

In the experimental model we have devised, mice were treated with CsA in doses ranging from 1.0 to 40.0 mg/kg/d to produce serum CsA levels falling within the "therapeutical window" generally used in BMT patients. These doses were not associated with signs suggestive of a significant nephrotoxicity in the recipient animals, as assessed by both the unchanged serum creatinine levels and the lack of histologic signs of renal toxicity. Although these animals did not present, in the steady-state condition, a significant reduction of either Hct or serum Epo levels, they were unable to produce the expected amount of Epo when challenged with an anemic stress. This finding suggests that the treatment with CsA impairs the ability of Epo-producing cells to cope with the greatly increased demand of the hormone due to the anemic condition, and/or that CsA affects the sensor mechanism linking Epo production to the hypoxic stress. Our study does not allow us to distinguish between these two pathogenetic mechanisms. However, it is of interest to note that the inadequate Epo production observed in this animal model is, from several points of view, very close to that described in BMT patients.

First, the parameters of the regression line correlating serum Epo levels and Hct in CsA-treated mice were significantly different from those obtained in normal mice with comparable degrees of anemia (Fig 2), at the same way as in BMT patients, when the latter were compared with both normal and anemic Second, we found no significant correlation between CsA dosage and the degree of Epo production impairment, because even relatively low CSA dosages were associated with a significant impairment of Epo production. Similarly, Beguin et al. were unable to correlate serum Epo with either blood CsA levels or the duration of CsA therapy in BMT patients. In another study, no correlation was found between CsA and serum Epo levels after renal transplantation. These observations seem to suggest that the reduction of Epo production by CsA is not simply due to a toxic effect, but might be the result of a somehow more specific inhibition. Finally, CsA-treated mice developed an impaired Epo production without any significant modification of creatinine levels, as has been observed in BMT patients. Again, the mechanism(s) underlying the effects of CsA on Epo-producing cells appears to be different from those causing the classical CsA-induced nephrotoxicity.

In conclusion, although the molecular basis of the inhibition of Epo-synthesizing cells by CsA remains to be elucidated, our data indicate that CsA may have a role in the impaired Epo production that has been reported to occur in patients undergoing BMT.

ACKNOWLEDGMENT

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REFERENCES


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