RESPONSE TO CYCLOSPORIN A AND rhG-CSF IN A CASE OF APLASTIC ANEMIA

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ABSTRACT
A 23-year-old male patient developed aplastic anemia and was treated with cyclosporin A and rhG-CSF. Bone marrow biopsy showed an improvement in cellularity with a recovery of all hematopoietic precursors after nine weeks of therapy. WBC increased after two weeks of treatment, mostly due to an increase in the absolute granulocyte cell count. Hb, RBC, Plts and reticulocytes showed an increase six weeks after the beginning of therapy. Of the serum cytokines, EPO levels progressively decreased, while sTfR increased in peripheral blood. A reverse correlation between blood neutrophil count and serum levels of G-CSF was observed, indicating an increased clearance of G-CSF. Finally, sIL-2R showed a rapid increase in the first week of treatment and prior to the increase in PMN cells. Thus, the use of cyclosporin A and rhG-CSF in our patient induced a complete recovery of hemopoiesis, and this may be explained by a synergic effect induced by the capacity of cyclosporin A to remove inhibitory factors and the stimulatory activity of rhG-CSF.

Key words: aplastic anemia, cyclosporin A, rhG-CSF
Cyclosporin A and G-CSF in aplastic anemia

The presence of erythrocytes, serum and urinary lysozymes, pharyngeal swab, FAN, anti-nDNA, anti-ENA antibodies, direct and indirect Coombs’. Bone marrow biopsy specimens revealed severe hypocellularity. He was treated with cyclosporin A (Sandimmun, Sandoz; 5 mg/kg/day p.o. adjusted on the basis of plasma levels; range 100-200 ng/mL) and rhG-CSF (Neupogen, Dompé; 300 µg/day s.c.). After nine weeks, bone marrow biopsy showed an improvement in cellularity with a recovery of all hematopoietic precursors. Cyclosporin A was stopped after 21 weeks and rhG-CSF was continued. At the 35th week, a bone marrow biopsy specimen revealed improved post-hypoplasia cellularity and the therapy was suspended. Subsequently, laboratory examinations confirmed good resolution of the hematopoietic defect that lasted more than 12 months.

Materials and Methods

Serum cytokine levels were determined using an ELISA method, before and after therapy was begun. The following cytokines were investigated: sIL-2R (T Cell Science; n.v. 503±279 U/mL), G-CSF (R&D System; n.v. 40.1±21.7 pg/mL), erythropoietin (R&D System; n.v. 9.9±3.3 mU/mL), sTfR (AMGEN; n.v. 2142±440 ng/mL).

Results

Blood counts prior to treatment and following therapy are shown in Table 1. A favorable response was observed six weeks into therapy, with an increase in absolute reticulocyte count, Hb, RBC, Plts and a progressive normalization of serum iron values. WBC increased after two weeks of therapy, mostly due to an increase in the absolute neutrophil count. Serum erythropoietin levels (Figure 1) were high before treatment and significantly decreased after six weeks of treatment (from 322 mU/mL to 185 mU/mL). sTfR (Figure 1) progressively increased (from 1660 mg/mL in the 6th week to 4125 mg/mL in

<table>
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<tr>
<th>Weeks</th>
<th>−1</th>
<th>2</th>
<th>6</th>
<th>10</th>
<th>14</th>
<th>21</th>
<th>29</th>
<th>35</th>
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<tbody>
<tr>
<td>RBC 10⁹/L</td>
<td>2.12</td>
<td>1.98</td>
<td>3.49</td>
<td>3.51</td>
<td>3.50</td>
<td>3.68</td>
<td>4.05</td>
<td>4.16</td>
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<tr>
<td>Hb g/dL</td>
<td>5.7</td>
<td>7.1</td>
<td>11.4</td>
<td>11.5</td>
<td>12</td>
<td>12.7</td>
<td>13.4</td>
<td>13.9</td>
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<tr>
<td>Ret %</td>
<td>0.8</td>
<td>0.8</td>
<td>1.7</td>
<td>4.4</td>
<td>3.5</td>
<td>1.9</td>
<td>2.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Reticulocytes 10⁹/L</td>
<td>19</td>
<td>17</td>
<td>61</td>
<td>151</td>
<td>119</td>
<td>69</td>
<td>108</td>
<td>78</td>
</tr>
<tr>
<td>WBC 10⁹/L</td>
<td>2.3</td>
<td>6.7</td>
<td>9.6</td>
<td>18.5</td>
<td>16.6</td>
<td>20.5</td>
<td>19.6</td>
<td>21.8</td>
</tr>
<tr>
<td>Neutrophils 10⁹/L</td>
<td>0.51</td>
<td>3.4</td>
<td>6.5</td>
<td>14.9</td>
<td>12.7</td>
<td>16.8</td>
<td>16.2</td>
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</tr>
<tr>
<td>Platelets 10⁹/L</td>
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<td>20</td>
<td>35</td>
<td>104</td>
<td>144</td>
<td>161</td>
<td>132</td>
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<td>Serum iron mg/dL</td>
<td>224</td>
<td>221</td>
<td>131</td>
<td>101</td>
<td>81</td>
<td>74</td>
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Figure 1. Behavior of sTfR, EPO, reticulocyte count and Hb during therapy with CyA and rhG-CSF.
the 10th week), starting after six weeks of therapy. After two weeks, a pronounced decrease in G-CSF serum levels (Figure 2) was noted (from 5121 pg/mL in the first week to 2029 pg/mL in the second), and continued progressively. sIL-2R levels increased to 976 U/mL after 3 weeks and then to 1650 U/mL after 35 weeks of treatment (Figure 2). They remained at higher values during the observation period.

Side effects consisted of a slight increase in serum creatinine levels, which improved when the serum cyclosporin A level was adjusted, gengival hyperplasia and an increase in blood lipid values.

**Discussion**

Evidence has been presented supporting immunosuppression of hematopoiesis by activated T-suppressor cells in some cases of AA.1 Subsequent laboratory data and clinical observations continue to make immune mediation of bone marrow failure an attractive hypothesis.1 ALG and ATG have been reported to be effective therapy for AA because of their immunosuppressive, immunomodulatory or indirect stimulatory effects on hematopoiesis.1,6 Recently, CyA was shown to induce a favorable response in some patients with AA.7 CyA selectively inhibits both antigen-induced CD4+ lymphocytes and the cell production of IL-2 and other cytokines. Moreover, it interrupts active immune responses and blocks lymphocyte cytokine secretion.1 Dysregulation in the cytokine network has been reported to be responsible for hematopoietic failure in patients with AA. High serum levels of some cytokines which are known to suppress hematopoietic stem cells have been described in untreated patients.5 Kojima detected normal or elevated production of growth factors (i.e. G-CSF, GM-CSF, IL-6) by marrow stromal cells in patients with AA.9 Because CyA and G-CSF combined may abolish myelopoiesis suppressive mechanisms, influence cytokine release and stimulate multipotential stem cells,3 we evaluated therapy-induced variations in the serum levels of some soluble factors. As reported,10 EPO values were higher before treatment, and decreased when erythropoiesis improved. A response to therapy was confirmed by the behavior of sTfR, a truncated circulating form of the entire cell-membrane transferrin receptor. Serum levels of sTfR have been demonstrated to correlate with erythroid activity in healthy subjects and in different types of anemias, and are a simple, rapid method for evaluating erythropoiesis in vivo.11 Our patient also presented increased serum sTfR and improvement in reticulocyte count and Hb values, confirming that sTfR is not only useful for early response detection but also for subsequent monitoring of erythropoiesis. Serum levels of...
G-CSF have been estimated in patients with various disorders (AA, CLL, CML), and high values were observed in patients with AA. A reverse correlation between blood neutrophil count and serum levels of G-CSF was also observed in our patient. A decrease in serum G-CSF levels has been reported during therapy, with increased neutrophil levels, indicating increased clearance of G-CSF.

sIL-2R, initially in the normal range, subsequently showed a rapid increase in the first week of treatment and prior to the increase in PMN cells. This is in accordance with Drege et al., who observed an increase in sIL-2R levels in vivo induced by G-CSF, even in the absence of peripheral blood leukocytes. This increase occurs independently of lymphocyte activation and may be explained by direct or indirect involvement of immature bone marrow cells in the release of sIL-2R. This hypothesis is consistent with results showing that low-affinity IL-2 receptors are expressed by early myeloid cells. However, elevated sIL-2R levels were found in clinical conditions with an increased number of immature hematopoietic cells. Thus, normal progenitor cells may display similar patterns of IL-2R expression upon exogenous stimulation with growth factors.

In our patient the recovery of erythropoiesis through modification of erythropoietin and sTfR levels occurred contemporarily with the increase in WBC. This may be explained by the synergic effect induced by the capacity of CyA to remove inhibitory factors together with the stimulatory activity of G-CSF.

In conclusion, the association of CyA and G-CSF was successful in this patient. Moreover, prospective controlled trials are needed to ascertain whether cyclosporin A and G-CSF represent optimal treatment for these patients, or whether the use of ALG may further improve the results.

References