Complete Remission in Severe Aplastic Anemia After High-Dose Cyclophosphamide Without Bone Marrow Transplantation

By Robert A. Brodsky, Lyle L. Sensenbrenner, and Richard J. Jones

Severe aplastic anemia (SAA) can be successfully treated with allogeneic bone marrow transplantation (BMT) or immunosuppressive therapy. However, the majority of patients with SAA are not eligible for BMT because they lack an HLA-identical sibling. Conventional immunosuppressive therapy also has major limitations; many of its remissions are incomplete and relapse or secondary clonal disease is common. Cyclophosphamide is a potent immunosuppressive agent that is used in all BMT conditioning regimens for patients with SAA. Preliminary evidence suggested that high-dose cyclophosphamide, even without BM, may be beneficial to patients with SAA. Therefore, 10 patients with SAA and lacking an HLA-identical sibling were treated with high-dose cyclophosphamide (45 mg/kg/d) for 4 consecutive days with or without cyclosporine. A complete response (hemoglobin level, >13 g/dL; absolute neutrophil count, >1.5 x 10^9/L; and platelet count >125 x 10^9/L) was achieved in 7 of the 10 patients. The one complete responders died from the acquired immunodeficiency syndrome 44 months after treatment with high-dose cyclophosphamide. The remaining patients are alive and in continuous complete remission, with a median follow-up of 10.8 years (range, 7.3 to 17.8 years). The median time to last platelet transfusion and time to 0.5 x 10^9 neutrophils/L were 85 and 95 days, respectively. None of the complete responders has relapsed or developed a clonal disease. These results suggest that high-dose cyclophosphamide, even without BMT, may be more effective than conventional immunosuppressive therapy in restoring normal hematopoiesis and preventing relapse or secondary clonal disorders. Hence, further studies confirming the efficacy of this approach in SAA are indicated. © 1998 by The American Society of Hematology.

A CQUIRED SEVERE aplastic anemia (SAA) is a rare hematopoietic disorder that can be successfully treated by bone marrow transplantation (BMT) or immunosuppressive therapy. BMT leads to complete hematopoietic recovery and cure of the disease in 60% to 80% of patients, with the major causes of failure being graft rejection and graft-versus-host disease. However, the majority of patients with SAA are not candidates for BMT because they lack an HLA-identical sibling. The majority (60% to 80%) of SAA patients treated with immunosuppression recover hematopoiesis sufficiently to be transfusion-independent and free of infection, although often not to completely normal blood counts. The combination of antithymocyte globulin (ATG) and cyclosporine appears to be the most effective form of immunosuppressive therapy. Patients with super severe aplastic anemia (SSAA), ie, SAA patients with an absolute neutrophil count (ANC) of less than 0.2 x 10^9/L at diagnosis, have a poorer response to immunosuppression than do patients with just SAA. A major limitation of immunosuppressive therapy is that up to 50% of successfully treated patients either relapse or develop late clonal diseases such as paroxysmal nocturnal hemoglobinuria (PNH), myelodysplastic syndrome (MDS), or acute leukemia.

Cyclophosphamide is a potent immunosuppressive agent and is used in all BMT conditioning regimens for patients with SAA. After preparation with cyclophosphamide, most allogeneic bone marrow grafts persist indefinitely; however, in several cases, complete autologous hematopoietic recovery has occurred. Case reports of 2 SAA patients achieving a complete hematologic remission with moderately high-dose cyclophosphamide (40 to 120 mg/kg) without BMT have also been described. These data suggest that cyclophosphamide, particularly in high doses, may be beneficial to patients with SAA even without allogeneic BMT. We treated 10 SAA patients lacking an HLA-identical sibling donor with high-dose cyclophosphamide. A complete hematologic remission was achieved in 7 patients, and, with a median follow-up of more than 10 years, no patient has relapsed or developed secondary clonal disease.

MATERIALS AND METHODS

Patients. Patients referred to the Johns Hopkins Oncology Center for the treatment of SAA between 1977 and 1986 were eligible for this study if they lacked an HLA-identical donor. No patient was excluded from the study. However, between 1980 and 1984, all patients with SAA and without an HLA-identical donor were entered on studies of either ALG followed by haploidentical BMT and androgens (15 patients) or ATG alone (5 patients). These patients have been previously reported. The last patient accrued to this study was treated at Wayne State University. SAA was diagnosed by standard criteria; less than 25% marrow cellularity and depression in two of three blood counts (reticulocytes, <40 x 10^9/L; platelets, <20 x 10^9/L; and an ANC <0.5 x 10^9/L). Aplastic anemia was considered severe if it met the criteria for severe disease and the ANC was less than 0.2 x 10^9/L. All patients gave informed consent for study participation approved by the institutional review boards of Johns Hopkins University and Wayne State University. Dose and treatment schedule. After confirmation of the diagnosis, patients were randomized to receive cyclophosphamide at 45 mg/kg/d intravenously (IV) or days 1 through 4, with or without cyclosporine. Patients randomized to receive cyclosporine received 5 mg/kg IV on days 4 through 9, 3.75 mg/kg/d IV on days 10 through 20, 2.5 mg/kg/d IV on days 21 through 27, and on days 28 through 32 both 1.5 mg/kg/d IV and 5 mg/kg/d orally. The patients then received cyclosporine at 10 mg/kg/d orally on days 33 through 56 and 7.5 mg/kg/d orally from days 57 through 100, after which the drug was discontinued. All patients received only one course of therapy. Response. A complete response required normalization of all blood counts (ie, hemoglobin level, >13 g/dL; ANC >1.5 x 10^9/L; and platelet count, >125 x 10^9/L). All others were classified as...
Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>ANC (×10^9/L)</th>
<th>Retics (×10^9/L)</th>
<th>Plts (×10^9/L)</th>
<th>Etiology</th>
<th>Previous Therapy</th>
<th>Duration of SAA Before CY (wk)</th>
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<tbody>
<tr>
<td>1</td>
<td>22/F</td>
<td>670</td>
<td>18</td>
<td>8</td>
<td>Benzene exposure</td>
<td>Androgens/steroids</td>
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<td>2</td>
<td>16/M</td>
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<td>3</td>
<td>19/M</td>
<td>40</td>
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<td>None</td>
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<td>2</td>
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<tr>
<td>5</td>
<td>16/M</td>
<td>310</td>
<td>14</td>
<td>10</td>
<td>Idiopathic</td>
<td>None</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>30/F</td>
<td>780</td>
<td>36</td>
<td>18</td>
<td>Idiopathic</td>
<td>None</td>
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<td>7</td>
<td>7/F</td>
<td>75</td>
<td>26</td>
<td>8</td>
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<td>3</td>
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<td>38/M</td>
<td>600</td>
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<td>10</td>
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<td>Steroids</td>
<td>40</td>
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<td>9</td>
<td>13/F</td>
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<td>26</td>
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<td>300</td>
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<td>10</td>
<td>Idiopathic</td>
<td>Androgens/steroids</td>
<td>8</td>
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</tbody>
</table>

Abbreviations: CY, cyclophosphamide; Retics, absolute reticulocyte count; Plts, platelet count.

nonresponders. Persistence of a transfusion requirement was evidence of no response.

RESULTS

Patient characteristics are shown in Table 1; no patients were excluded from analysis. The median age (19 years; range, 7 to 38 years) was similar to that of other studies of SAA. Of the 10 patients, 5 had SSAA and 5 had SAA. Most of the patients were newly diagnosed; the median duration of disease before treatment with high-dose cyclophosphamide was 1 month (range, 2 weeks to 1 year), and only 3 patients had received any prior therapy for their SAA.

A complete response was achieved in 7 of the 10 patients; the majority of patients with both SAA (4/5) and SSAA (3/5) were complete responders (Table 1). An ANC of 0.5 × 10^9/L was reached at a median of 95 days (range, 34 to 201 days), and the last platelet transfusion was at a median of 85 days (range, 35 to 151 days; Table 2). The use of cyclosporine did not appear to influence whether the patients were able to achieve a complete response, with 5 of 7 patients treated with cyclophosphamide alone and 2 of 3 patients treated with cyclophosphamide plus cyclosporine attaining a complete response. None of the 7 complete responders has relapsed or developed a clonal disease, with a median follow-up of 10.8 years (range, 7.3 to 17.8 years); 6 of the 7 are alive and remain in complete remission with completely normal blood counts, and 1 patient became infected with the human immunodeficiency virus from a blood transfusion and died of acquired immunodeficiency syndrome (AIDS) 44 months after treatment (Table 2). Only 1 patient (in remission for >10 years) has an elevated mean corpuscular volume (MCV; 105 μm²; normal range, 80 to 100 μm²), which has been reported to be predictive for the development of late clonal disorders in SAA patients treated with immunosuppression. Dysplastic changes in the remission bone marrows (especially in the monocytic and megakaryocytic lineages) may also be associated with the development of late clonal disorders, but were not present in any of the patients.

High-dose cyclophosphamide was generally well-tolerated, with toxicities similar to those seen with autologous BMT. All acute side-effects resolved completely, with no evidence of late sequelae (although gonadal function has not been extensively evaluated). All patients developed febrile neutropenia requiring combinations of antibiotics. Transient blood cell count; Hgb, hemoglobin.

Table 2. Hematologic Response to High-Dose Cyclophosphamide

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Treatment (date)</th>
<th>Status</th>
<th>ANC of WBC (×10^9/L)</th>
<th>Last Plts Transfusion</th>
<th>ANC of WBC (×10^9/L)</th>
<th>Hgb (g/dL)</th>
<th>Plts (×10^9/L)</th>
<th>MCV (μm²)</th>
<th>Date</th>
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<tr>
<td>1</td>
<td>CY (6/77)</td>
<td>CCR</td>
<td>95</td>
<td>35</td>
<td>5.7</td>
<td>2.0</td>
<td>13.8</td>
<td>322</td>
<td>99</td>
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<td>2</td>
<td>CY (1/78)</td>
<td>CCR</td>
<td>201</td>
<td>95</td>
<td>5.9</td>
<td>1.9</td>
<td>14.6</td>
<td>295</td>
<td>98</td>
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<tr>
<td>3</td>
<td>CY (8/78)</td>
<td>NR</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>CY (4/84)</td>
<td>CCR</td>
<td>74</td>
<td>78</td>
<td>7.2</td>
<td>3.7</td>
<td>13.8</td>
<td>310</td>
<td>92</td>
</tr>
<tr>
<td>5</td>
<td>CY/CSA (7/84)</td>
<td>CR*</td>
<td>117</td>
<td>151</td>
<td>4.2</td>
<td>2.3</td>
<td>15.1</td>
<td>140</td>
<td>94</td>
</tr>
<tr>
<td>6</td>
<td>CY/CSA (11/84)</td>
<td>CR</td>
<td>47</td>
<td>85</td>
<td>5.8</td>
<td>2.8</td>
<td>14.6</td>
<td>166</td>
<td>105</td>
</tr>
<tr>
<td>7</td>
<td>CY (2/85)</td>
<td>CCR</td>
<td>96</td>
<td>125</td>
<td>5.4</td>
<td>2.6</td>
<td>13.8</td>
<td>182</td>
<td>96</td>
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<tr>
<td>8</td>
<td>CY/CSA (2/86)</td>
<td>NR</td>
<td>167</td>
<td>—</td>
<td>—</td>
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</tr>
<tr>
<td>10</td>
<td>CY (9/87)</td>
<td>CCR</td>
<td>34</td>
<td>35</td>
<td>8.4</td>
<td>5.1</td>
<td>13.3</td>
<td>202</td>
<td>99</td>
</tr>
</tbody>
</table>

Abbreviations: CY, cyclophosphamide; CSA, cyclosporine; CCR, continuous complete remission; NR, no response; Plts, platelet; WBC, white blood cell count; Hgb, hemoglobin.

* Died of acquired immunodeficiency syndrome 44 months after treatment.
hepatitis, presumed to be viral in origin, developed in 4 patients; 2 patients experienced self-limited renal dysfunction, although 1 required short-term dialysis for a creatinine that reached a peak of 8.3 mg/dL. Hypertension requiring treatment developed in 2 patients who received cyclosporine, and transient congestive heart failure requiring diuretics occurred in 1 patient. No patient developed hemorrhagic cystitis.

There were 3 patients who did not respond to therapy and subsequently died of their disease. One nonresponder recovered his ANC to greater than 0.5 × 10^9/L, but died 31 months after treatment from an intracerebral hemorrhage. The 2 other deaths occurred within 6 months of therapy and were secondary to intracerebral hemorrhage and sepsis, respectively.

**DISCUSSION**

We found that high-dose cyclophosphamide without BMT produced long-term survival and normal hematopoiesis in patients with SAA. The response rate in our series is comparable with response rates seen after allogeneic BMT2-7 or conventional immunosuppressive therapy with antithymocyte globulin and cyclosporine.6,11. A previous report suggested a lower response rate for SAA to cyclophosphamide.12 However, most patients in the previous report received relatively low doses of cyclophosphamide and the highest dose was only two-thirds of the dose used in our study.22

Like BMT, high-dose cyclophosphamide in our series produced complete hematopoietic recovery in all responders and was not associated with relapse or late clonal diseases despite at least 7 years of follow-up in all patients. In contrast, conventional immunosuppressive therapy frequently fails to restore normal hematopoiesis and relapse is common.6,10,11 Furthermore, conventional immunosuppressive therapy is also associated with a significant risk of late clonal diseases. Tichelli et al.17 reported a 42% cumulative risk of developing PNH or MDS at 10 years after therapy with antithymocyte globulin. The European Group for Bone Marrow Transplantation (EBMT) reported an 18.8% cumulative incidence of cancer at 10 years after immunosuppressive therapy16; however, they did not include PNH in their definition of malignancy. The EBMT previously reported that the incidence of PNH after immunosuppressive therapy for SAA is at least 10%.14 The mean interval to the development of PNH and MDS after immunosuppressive therapy is 3.0 and 4.6 years, respectively.14 Recent studies using both antithymocyte globulin and cyclosporine for the treatment of SAA report a lower incidence of late clonal diseases, but these studies still have a relatively short follow-up.6,10,11

As with other forms of immunosuppression, some patients with SAA failed to respond to high-dose cyclophosphamide. This may result from a failure to abrogate immunologic-mediated hematopoietic suppression (analogous to graft rejection after allogeneic BMT2,6,20) or a deficiency of normal host stem cells capable of reestablishing hematopoiesis. Although high-dose cyclophosphamide may be somewhat more toxic than conventional immunosuppression, there were no deaths related to the procedure and all acute side-effects were self-limited in this series. Moreover, the median time to an ANC of 0.5 × 10^9/L and transfusion-independence in this series (day 95 and 85, respectively) is comparable to that seen with conventional immunosuppression,10 even though no patient in our series received growth factor support.

HLA-identical sibling BMT is currently the treatment of choice for young patients with SAA, primarily because of the lower risk of relapse and late clonal disorders when compared with patients treated with immunosuppression. Our data suggest that high-dose cyclophosphamide without BMT may also effectively restore normal hematopoiesis and prevent relapse or secondary clonal disorders. Although the beneficial effects of high-dose cyclophosphamide may just be a consequence of its intensive immunosuppressive activity, it is likely that the potent cytotoxic properties of cyclophosphamide are also important; this could have important implications regarding the pathophysiology of SAA. It is not clear why aplastic anemia is a premalignant disorder.25,27,28 Damage to hematopoietic stem cells may lead to an autoimmune response directed against the bone marrow.29 In addition, sublethal damage to a stem cell may produce genetic mutations that give it a growth advantage. Over time, an abnormal clone arising from a slowly proliferating, transformed stem cell could become dominant. Cyclophosphamide not only causes profound immunosuppression, but also has the potential to destroy a minor population of damaged stem cells with the capacity to develop into MDS, PNH, or acute leukemia.

Although encouraging, these results remain preliminary. The study was small and the patients were all relatively young. Hence, further studies with high-dose cyclophosphamide need to be performed. Nevertheless, our data suggest that this approach should be considered as an alternative to immunosuppressive therapy in patients who lack an HLA-identical sibling.

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