Antithoracic Duct Lymphocyte Globulin Therapy of Severe Aplastic Anemia

By Bruce Camitta, Richard J. O'Reilly, Lyle Sensenbrenner, Joel Rappeport, Richard Champlin, Kris Doney, Charles August, Raymond G. Hoffmann, Dahlia Kirkpatrick, Robert Stuart, George Santos, Robertson Parkman, Robert Peter Gale, Rainer Storb, and David Nathan

We performed a prospective randomized trial of antithoracic duct lymphocyte globulin (ATDLG), HLA-haploididentical marrow, and androgen (regimen ABA) versus androgen alone (concurrent STANDARD care controls) in 42 newly diagnosed individuals with severe aplastic anemia. ABA patients also were matched with patients from our preceding study (historical STANDARD care controls). Supportive care and pretreatment patient characteristics were the same in all groups. By life table analysis, 76% of patients receiving ABA are alive at 2 yr compared to 31% of the concurrent control group (p < 0.002 versus ABA) and 19% of the historical controls (p < 0.0001 versus ABA) given STANDARD care. ABA patients had greater hematologic improvement than either control group (p < 0.001). However, improvement with ABA was often incomplete. Toxicity of ATDLG was considerable but manageable. Further studies to determine the mechanism of action and active component(s) of ABA are indicated.

Aplastic Anemia is characterized by peripheral blood pancytopenia and bone marrow hypocellularity. Duration of survival varies inversely with the severity of hematologic depression at diagnosis.4-6 Despite improvements in supportive care, less than 25% of patients with severe marrow aplasia survive.3,4 Although anabolic hormones (androgens) may benefit individual patients, use of these drugs has not improved prognosis in large groups of patients with severe aplastic anemia.4

There is increasing evidence that bone marrow damage in aplastic anemia can be initiated or perpetuated by immunologic mechanisms.5,6 These data resulted in trials of immunosuppressive therapy for marrow aplasia. Antilymphocyte preparations have been studied most extensively in this regard. Mathé et al. reported survival with partial autologous marrow recovery in 2 of 4 patients with marrow aplasia who were treated with antilymphocyte serum and HLA-nondonidential marrow.7 Subsequent studies, especially those of Speck, also suggested that antilymphocyte preparations improved survival of patients with aplastic anemia.8-15 However, these trials are difficult to interpret because of the variable severity of patients' marrow aplasia, inconsistent concomitant use of androgens and HLA-haploidentical marrow, and possible bias introduced by selection of patients for treatment who already had prolonged survival.

Based on the above considerations, the Aplastic Anemia Study Group conducted a prospective randomized study of antithoracic duct lymphocyte globulin (ATDLG), HLA-haploidentical marrow, and androgen (regimen ABA) versus androgen (STANDARD care) in patients with newly diagnosed severe aplastic anemia. Our data suggest that ABA therapy improves both survival and hematologic recovery when compared to STANDARD therapy for severe aplastic anemia.

MATERIALS AND METHODS

Patient Selection

Patients with severe aplastic anemia diagnosed less than 30 days prior to referral were eligible for this study. Severe aplastic anemia was defined as: (1) a markedly hypoplastic marrow (<25% of normal cellularity) or a moderately hypocellular marrow (25%-50% of normal cellularity with <30% hematopoietic cells), and (2) at least 2 of the following 3 peripheral blood criteria—granulocytes <500/µl, platelets <20,000/µl, corrected reticulocytes <1% (reticuloocyte x hematocrit/40).4 Following referral, patients were observed for 10 days to detect underlying disease, to establish baseline blood counts, to permit spontaneous improvement, and to complete histocompatibility studies. During this interval, prednisone (10 mg/sq m) could be given to decrease vascular fragility or to unmask underlying leukemia.18 Patients with Fanconi's anemia,
malignancy, or other underlying illnesses were excluded from this trial. Patients less than 50 yr old who had a histocompatible sibling donor were offered bone marrow transplantation.

Randomization

Informed consent was obtained from the patient or appropriate family members prior to randomization, using forms approved by institutional research committees. Randomization was performed using a table of random numbers. Two patients were assigned to the ABA treatment (horse anti-human thoracic duct lymphocyte globulin, HLA-haploidentical bone marrow, androgen, and supportive care) for each patient assigned to receive standard medical care (androgen and supportive care). This unbalanced randomization and the use of additional matched historical controls from Aplastic Anemia Study I were designed to maximize the number of patients who would receive potentially effective new therapy.

Patient Care

Patients in all groups received standard optimal medical care. Platelet counts were maintained >10,000/µl with platelet transfusions. HLA-matched platelets were not utilized until patients were refractory to random donor platelets. Other blood products were given as necessary. Prophylactic antibiotics and special isolation procedures were not utilized. All patients received oral androgen (oxymetholone, 3–5 mg/kg/day) because hormonal therapy might benefit individual patients receiving standard medical care and because it had been suggested that hormonal therapy was necessary for success of ATDLG therapy. Anti-human thoracic duct lymphocyte globulin (ATDLG) administration was patterned after the protocol of Speck. ATDLG was supplied by the Swiss Serum Institute. The dose was 40 mg/kg lean body weight/day for 4 consecutive days. Prior to the initial infusion, patients were skin tested with 0.1 ml of a 1:1,000 dilution of ATDLG in normal or half-normal saline. A positive systemic reaction (urticaria, dyspnea, hypotension, anaphylaxis) mandated removal from the protocol. No reactions were seen. The total daily dose of ATDLG was diluted in several 250–500-ml bottles of normal or half-normal saline. Bicarbonate was added at some centers to produce a more neutral pH. Five to ten percent of the daily dose was given in the first hour. The remaining drug was given over 5–11 hr at a rate adjusted to avoid hypotension. Premedication with diphenhydramine, meperidine, or corticosteroid (7) therapy prior to referral; 4 of 13 patients randomized to ABA had received some androgen (3) or corticosteroid (7) therapy prior to referral; 4 of 13 patients randomized to standard care had received prior androgen (2) or corticosteroid (4) treatment. Twenty-five of the 29 ABA patients could be matched (for age, sex, severity of disease, and treatment) with patients from our preceding study. Table 1 shows that there were no differences in all respects listed. In addition, 8 of 29 patients randomized to ABA had received some androgen (3) or corticosteroid (7) therapy prior to referral; 4 of 13 patients randomized to standard care had received prior androgen (2) or corticosteroid (4) treatment. Twenty-five of the 29 ABA patients could be matched (for age, sex, severity of disease, and treatment) with patients from our preceding study.

Criteria for Response

Complete response was defined as the return of all blood counts to normal values. Partial response meant improvement so that the patient's hematologic status no longer qualified as severe and blood product transfusions were not required. Patients randomized to ABA who did not improve within 4 mo were eligible to receive alternate therapy. Patients randomized to standard care who did not improve within 4 mo were eligible to receive ABA.

Statistical Considerations

Product limit estimates of the survival time distributions were plotted according to the method of Kaplan and Meier. A Wilcoxon–Gehan test was used to evaluate the effect of single factors on survival. A proportional hazards regression model was used to evaluate possible prognostic factors simultaneously.

RESULTS

Between January 1980 and March 1982, 42 patients with newly diagnosed severe aplastic anemia were entered on the study. Pretreatment patient characteristics are presented in Table 1. The ABA and concurrent STANDARD treatment groups were comparable in all respects listed. In addition, 8 of 29 patients randomized to ABA had received some androgen (3) or corticosteroid (7) therapy prior to referral; 4 of 13 patients randomized to standard care had received prior androgen (2) or corticosteroid (4) treatment. Twenty-five of the 29 ABA patients could be matched (for age, sex, severity of disease, and treatment) with patients from our preceding study. Table 1 shows that there were no differences in presenting characteristics between patients who received ABA and the historical STANDARD treatment controls.

Results of treatment are summarized in Table 2 and Fig. 1. The ABA regimen resulted in increased survival when compared to concurrent STANDARD therapy (p < 0.002), historical STANDARD therapy (p <

Table 1. Pretreatment Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>ABA</th>
<th>Standard (Concurrent)</th>
<th>Standard (Historical)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>29</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>Age (yr)*</td>
<td>16 (1-62)</td>
<td>19 (5-60)</td>
<td>13 (1-65)</td>
</tr>
<tr>
<td>Male:female</td>
<td>15:14</td>
<td>7:6</td>
<td>15:10</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>21</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Drug</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Uncertain</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Months symptoms to diagnosis*</td>
<td>&lt;1 (0-5)</td>
<td>&lt;1 (0-7)</td>
<td>&lt;1 (0-5)</td>
</tr>
<tr>
<td>PMN/µl*</td>
<td>300 (0-1,560)</td>
<td>126 (0-1,104)</td>
<td>240 (0-2,400)</td>
</tr>
<tr>
<td>Platelets/10³/µl*</td>
<td>6 (2-15)</td>
<td>10 (3-19)</td>
<td>8 (1-24)</td>
</tr>
<tr>
<td>Reticulocyte (%)†</td>
<td>0.2 (&lt;0.1-1.4)</td>
<td>0.2 (&lt;0.1-6)</td>
<td>0.2 (&lt;0.1-2.0)</td>
</tr>
</tbody>
</table>

*Values given as median (range).
†Singly corrected for the hematocrit.
logic improvement. Patients who received ABA experienced significantly more hematologic improvement than those in either STANDARD treatment group \((p < 0.001; \text{chi-square test of response by therapy})\).

Six ABA patients had significant deviations from protocol. Two received only two doses of ATDLG because of severe anaphylactic reactions. HLA-haploidentical marrow was not given to either of these individuals. Both patients are alive—one with a partial response, the other with transient improvement only. Two patients had delayed administration of the HLA-haploidentical marrow. One, delayed 2 wk because of marked lymphocytosis, died without improvement; the other, delayed for 4 wk because the donor was pregnant, has achieved a partial remission. Two patients had androgen inadvertently omitted for 2 mo. One of these individuals died after 3 mo without improvement; in the other, a good partial response began 2 mo after initiating hormone therapy. Numbers are too small to assess the effects of any of the above protocol deviations on hematologic recovery.

Responses to ABA usually began 4–10 wk after administration of ATDLG. Increased bone marrow cellularity was followed rapidly by reticulocytosis and increases in polymorphonuclear cells (PMNs). Platelet increases usually lagged 1–2 mo behind rises in other peripheral blood cells. The median time after ATDLG until PMNs were >500/μl was 10 wk (range 0–24 wk). The median time after ATDLG until platelets were >40,000/μl was 19 wk (range 2–52 wk).

Incomplete hematologic recovery was common in all treatment groups. In responding survivors who had received ABA, current hemoglobin values are 6.6–16.0 g (median 13.2), PMN counts range from 500 to 6,000/μl (median 2,000), while platelet counts/μl are 16–198/μl (median 92). Although peripheral blood counts frequently decreased slightly as androgens were tapered, only 2 responding patients relapsed to severe status.

Toxicity during infusion of ATDLG is summarized in Table 3. Most complications were managed by slowing the ATDLG infusion, frequent transfusions, or medications (primarily antihistamines or corticosteroids). The one death during ATDLG administration was due to pseudomonas sepsis. In retrospect, this patient probably was infected prior to beginning therapy and should have had ATDLG infusion delayed. In seven informative patients tested, karyotypic analyses failed to document donor cells in blood or marrow samples taken 2 or more weeks after marrow infusion. Graft-versus-host disease was not observed.

As in previous studies,21 one-third of patients who received androgen developed abnormal liver function tests. Most abnormalities were transient and mild.

---

### Table 2. Maximum Responses of Patients by Initial Therapy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Complete</th>
<th>Partial</th>
<th>None</th>
<th>Died</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABA</td>
<td>29</td>
<td>5</td>
<td>15†</td>
<td>2</td>
</tr>
<tr>
<td>STANDARD (concurrent)</td>
<td>13</td>
<td>1</td>
<td>2</td>
<td>1*</td>
</tr>
<tr>
<td>STANDARD (historical)</td>
<td>25</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Data as of 12/1/82. See text for definitions of response.

*Withdrawn after 5 mo without response; received ABA with rapid partial response.

†Two of these patients relapsed to severe status; one was retreated with ATDLG but did not respond.

---

### Fig. 1. Kaplan-Meier plot of survival according to treatment.

Data as of 12/1/82. (A) ABA versus concurrent STANDARD treatment. (B) ABA versus concurrent and historical STANDARD treatment. Symbols (○, ■) indicate length of follow-up of survivors.
Two patients required discontinuation of androgen because of severe, recurrent hepatocellular dysfunction. No cases of peliosis hepatis, hepatic adenoma, or other tumors were recognized during this study.

Severity of pancytopenia, age (Table 4), sex, race, etiology of aplasia, prior therapy, and duration of symptoms did not predict response to ABA therapy. In our previous study, a high mean cell volume (MCV) and idiopathic disease were both associated with improved response to standard therapy. There was no apparent relationship of these parameters to responsiveness to ABA.

**DISCUSSION**

Treatment with antithoracic duct lymphocyte globulin, HLA-haploidentical marrow, and androgen decreases mortality in patients with severe aplastic anemia when compared to standard medical management. Undoubtedly, the antilymphocyte preparation is essential for this effect. The roles of androgen and haploidentical marrow are less well defined. Androgen therapy does not improve the prognosis of severe aplastic anemia treated by standard medical therapy. In a recent survey, androgens did not appear to increase the response rate to immunosuppressive therapy of severe marrow aplasia. Nevertheless, some workers feel that these hormones are important for the success of antilymphocyte globulin therapy. Similarly, trials of antilymphocyte globulin with or without HLA-haploidentical marrow have given equivalent results. Mathé claimed that hematologic recovery occurred only in patients who evidenced transient marrow engraftment. However, in the UCLA controlled study, 11 of 21 patients with moderate or severe aplastic anemia improved within 3 mo of receiving antithymocyte globulin plus supportive care; no improvement was noted within 3 mo in 21 patients receiving supportive care alone. Neither group received marrow or androgen. In Speck's controlled study, survival following antilymphocyte globulin plus androgen therapy of severe aplastic anemia was not improved in patients who also received haploidentical marrow; hematologic improvement was slightly greater in the patients who received marrow. If androgen and haploidentical marrow are to be used in the future, their effectiveness should be confirmed by prospective controlled studies.

Most of our patients received corticosteroids during ATDLG or for treatment of serum sickness. Doses were not uniform. Only five patients received more than 0.5–2.0 mg/kg/day of prednisone or its equivalent. Low to moderate doses of corticosteroids do not improve prognosis in aplastic anemia. High-dose methylprednisolone has been reported to cause remissions in 50% of patients with aplastic anemia. The latter trial was uncontrolled, many patients received additional treatments, and in vitro treatment with prednisone did not improve marrow colony-forming capacity. In vitro marrow growth was increased by corticosteroids in another report of one aplastic patient. Recently, Gratwohl reported improvement in 7 of 10 patients with severe aplastic anemia who were treated with high-dose methylprednisolone in addition to antilymphocyte globulin, HLA-haploidentical marrow, and androgen. Controlled studies of the role of corticosteroids in conjunction with ABA therapy are needed.

Hematologic recovery following ABA was usually incomplete. This may reflect permanent damage to hematopoietic stem cells or to components of the marrow microenvironment. Alternatively, incomplete recovery may reflect persistent marrow suppression. Similar residual marrow damage has been described in busulfan-induced murine aplastic anemia and in survivors of severe aplastic anemia treated by immunosuppression or by standard medical means. Persistent marrow damage may increase a patient’s risk of developing further marrow aplasia, paroxysmal nocturnal hemoglobinuria, or leukemia. Although hematologic improvement has been stable in all but two of our patients, more prolonged follow-up is indicated.

We were unable to identify factors that predicted responsiveness to ABA in our patients with newly diagnosed severe aplastic anemia. This reflects, at least in part, the availability of effective therapy.
Champlin suggested that a prolonged interval from diagnosis decreased the success of antilymphocyte globulin therapy. In contrast, Gluckman's survey suggested that a prolonged interval from diagnosis to treatment increased the response rate to antilymphocyte globulin therapy. Our results do not resolve this issue. However, since the highest mortality in severe aplastic anemia treated by standard medical therapy occurs within 4-5 mo of diagnosis (Fig. 1), delay of ABA once underlying disease has been ruled out would appear to be imprudent.

Patients with less severe marrow damage might respond more frequently to ABA than those with severe aplastic anemia. Our study did not address this issue. Use of ABA in mild aplastic anemia must balance the morbidity of ABA, potential therapeutic benefits, and the course of the disease with standard medical management.

The mechanism of action of ATDLG is not known. An obvious possibility is the elimination of T cells or non-T-cells that may suppress normal marrow proliferation. However, suppressor cells have been detected in only a minority of untransfused patients with severe aplastic anemia. ATDLG also might act by stimulating hematopoiesis or by preventing sensitization to platelets or other blood products. Five of 29 patients treated with ABA developed sensitization to random donor platelets (at <1, 3, 3, 4, and 6 mo); 3 of 13 patients receiving concurrent STANDARD treatment became sensitized (at <1, 5, and 6 mo). Seven of the 9 deaths in the concurrent STANDARD treatment group occurred without evidence of sensitization to random donor platelets. Thus, prevention of sensitization does not appear to be a major contributing factor to successful ABA therapy.

Antilymphocyte globulins contain multiple specificities. The effective antibody for treatment of aplastic anemia may not be present in all presentations. Determination of the critical therapeutic target antigen(s) is necessary. This might ultimately allow production of more specific, less toxic, monoclonal antibodies. ATDLG toxicity also could be decreased further by the development of human (rather than heterologous) antilymphocyte preparations.

Histocompatible bone marrow transplantation has been the treatment of choice for severe aplastic anemia. Survival rates of more than 80% for untransfused patients treated with cyclophosphamide, 70% for transfused patients who received cyclophosphamide plus supplemental buffy coat cells, and 50% for transfused patients who received cyclophosphamide alone have been reported in large series of transplanted individuals. Peripheral blood hematologic recovery is complete in most marrow transplant survivors, but in vitro marrow colony-forming capacity may be decreased in some. Of the survivors, 20%–50% develop chronic graft-versus-host disease. During 1980–1981, 70% of patients with severe aplastic anemia who received HLA-identical bone marrow transplants at centers in the current study survived; chronic graft-versus-host disease occurred in one-third of the survivors.

Several authors have suggested that antilymphocyte preparations are more effective than bone marrow transplantation for treatment of severe aplastic anemia. However, these comparisons were uncontrolled or marrow transplant results were significantly worse than those referred to above. We suggest that controlled comparisons at this time would show ABA and histocompatible bone marrow transplantation to be equivalent with respect to survival. Hematologic recovery is usually less complete following ABA, but patients treated with ABA do not develop graft-versus-host disease or its sequelae. In patients older than 30, histocompatible bone marrow transplantation has not provided a significant survival advantage when compared to supportive care alone. In contrast, in our study, ABA was effective in all age groups (Table 4). ABA also should be considered for multiply transfused patients with severe aplastic anemia. This latter group is at significant risk for transplant rejection or from complications of regimens currently used to prevent rejection. ABA therapy improves survival and the frequency of hematologic recovery in severe aplastic anemia. However, therapy of aplastic anemia should not be considered a static issue. Promising new treatments should be evaluated prospectively for their merits.

REFERENCES

Antithoracic duct lymphocyte globulin therapy of severe aplastic anemia

B Camitta, RJ O'Reilly, L Sensenbrenner, J Rappeport, R Champlin, K Doney, C August, RG Hoffmann, D Kirkpatrick, R Stuart, G Santos, R Parkman, RP Gale, R Storb and D Nathan