A Multicenter Trial of Antithymocyte Globulin in Aplastic Anemia and Related Diseases


One hundred fifty patients with bone marrow failure were treated in three groups with antithymocyte globulin (ATG; Upjohn, Kalamazoo, MI) in a multicenter trial. Patients were assessed at 3, 6, and 12 months after initiation of treatment by three criteria: transfusion independence, clinical improvement, and blood counts. Group I consisted of 77 patients with acute severe aplastic anemia, randomized to receive either ten or 28 days of ATG. There was no significant difference between the two arms of this protocol: 47% of all patients were clinically improved and 31% were transfusion independent at 3 months. Of the severely affected patients, 27% died before 3 months; most deaths occurred early in treatment. Factors associated with survival in severely affected patients included male sex, age <40 years, absolute neutrophil count >200/µL, and idiopathic etiology. Neutrophil counts generally increased by 8 weeks after treatment, but patients continued to show improvement to 1 year posttreatment. In Group II, 44 patients with moderate or chronic severe aplastic anemia were randomized to receive either ten days of ATG or 3 months of high-dose dexamethasone. No patient initially treated with androgens recovered, but 28% of ATG-treated cases achieved transfusion independence at 3 months. Group III consisted of patients with a variety of bone marrow failure syndromes. Patients with pancytopenia and cellular bone marrow showed response rates similar to those of patients with chronic or moderate aplastic anemia.

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HE USE OF ANTILYMPHOCYTE SERA in aplastic anemia began with the observation by Mathé et al that some patients recovered autologous hematopoietic function after unsuccessful bone marrow transplantation, presumably as a result of immunosuppression from the conditioning regimen of antithy rocytic duct lymphocyte globulin (ALG).1 Large trials in Europe have suggested that ALG is as effective as bone marrow transplantation in prolonging survival in severe aplastic anemia.2-4 Smaller American controlled studies have shown antithymocyte globulin (ATG) to be superior to expectant treatment by observation with transfusion support alone in improving hematopoiesis,5 and ALG to be superior to androgens6 in improving hematopoiesis. However, clinical response rates, as measured by different criteria over variable time periods, have ranged from 14% to 85%.7 Most studies have reported hematologic improvement in 40% to 60% of patients treated.8

In this study, 13 centers in the eastern United States cooperated in a trial of ATG in aplastic anemia and other bone marrow failure states. The objectives of this clinical investigation were: to determine a realistic response rate to commercially available ATG in a variety of institutional settings; to compare a ten-day regimen with a 28-day regimen (about a twofold difference in total ATG dose) in patients with acute severe aplastic anemia; to compare ATG with conventional androgen therapy in aplastic anemia; and to test the effectiveness of ATG in other bone marrow failure states possibly to aplastic anemia.

MATERIALS AND METHODS

Patient Selection

All patients were treated under a uniform protocol approved by the institutional review boards of each separate institution. Patients with HLA-identical siblings were offered bone marrow transplantation. There were no age restrictions. Patients with anemia secondary to radiation, cytotoxic chemotherapy, neoplastic disease, storage disease, or nutritional deficiency were excluded. Patients with concurrent diseases likely to lead to death within seven to ten days and patients obviously moribund also were excluded. Patients with a history of severe reaction to horse serum or systemic reaction during skin testing with ATG were not treated. Three groups of patients were treated (Fig I). Group I (N = 77) consisted of patients with acute severe aplastic anemia. Acute was defined as <3 months from time of diagnosis by bone marrow biopsy to treatment initiation. Severity was defined by the criteria of Camitta et al,8 depression in two of three blood counts (reticulocytes <40,000/µL, platelets <20,000/µL, neutrophils <500/µL) in the presence of a hypocellular bone marrow biopsy (<25% cellularity or <50% cellularity and <30% hematopoietic cells) without significant fibrosis. Bone marrow biopsies were required to be >1 cm in length and to be reviewed by two hematologists. Group II (N = 44) comprised patients with aplastic anemia with a more favorable prognosis than acute severe disease in whom randomization between ATG and androgen therapy could be ethically justified. Group II contained patients with chronic (>3 months between time of diagnosis by bone marrow biopsy and treatment) severe aplastic anemia and moderate aplastic anemia. Moderate disease was defined as failure to meet the criteria for severe disease but with at least two diminished blood counts ( reticulocytes <40,000/µL, platelets <40,000/µL, neutrophils <1,500/µL) with a hypocellular bone marrow biopsy. Of the patients in whom such a history could be obtained, 20 had received some androgen treatment prior to entry.
into the protocol, and 19 had never been treated with androgens; 23 of these patients were treated initially with ATG, 16 with nandrolone decanoate. Group III (N = 29) contained patients with a variety of bone marrow failure syndromes who were refractory to conventional therapy: pancytopenia with cellular bone marrow, pure red cell aplasia, amegakaryocytic thrombocytopenia, pure white cell aplasia, cyclical neutropenia, myelofibrosis, and paroxysmal nocturnal hemoglobinuria.

Twenty-two patients from groups I and II, treated at a single center, have been reported separately. Protocol Outline and Randomization

Randomization was performed separately for each institution in a blocked manner to ensure balance over time. In addition, random block sizes of four and six were used to prevent determination of the next assignment. Because of the apparent effectiveness of ATG in other studies, all patients with severe disease and a correspondingly poor prognosis received ATG. Group I patients were randomized to receive either ten or 28 days (14 days of daily therapy followed by 14 days of alternate-day therapy) of ATG. To examine the effectiveness of ATG compared to conventional therapy, group II comprised patients with a more favorable prognosis who either had moderate aplasia of any duration or severe disease and who had survived for >3 months. Because of uncertainty as to what constitutes an "adequate" trial of androgens, no attempt was made to correlate responsiveness with previous therapy. Group II patients were randomized to receive either ATG for ten days or nandrolone decanoate 5 mg/kg/wk by intramuscular injection for 11 weeks. Patients who had failed to respond to initial therapy (ATG or androgen) at 3 months were then treated with the crossover therapy (androgen or ATG). All Group III patients received ATG for ten days.

Patient Care

A single lot of ATG was assigned to this study (lot no. 17924; Upjohn, Kalamazoo, MI). ATG was administered at a dose of 15 mg/kg/d. The total daily dose was diluted in 500 mL to 1,000 mL of physiologic saline and administered intravenously (IV) over four to five hours with a microaggregate in-line filter. Skin testing was performed before therapy: 0.1 mL of a 1:1,000 dilution of ATG in saline was injected intradermally. A severe local reaction (>5 cm induration or erythema) or an immediate systemic reaction warranted exclusion from treatment. Methylprednisolone was administered concurrently as 40 mg within the ATG infusion bottle. The remainder was administered orally in four daily divided doses to a total of 1 mg/kg/d (maximum of 56 mg daily). Methylprednisolone was increased to a maximum of 64 mg daily for treatment of serum sickness. Antihistamines and meperidine were administered for allergic symptoms.

Supportive care was standardized among the participating institutions. During periods of ATG infusion, platelet counts were maintained at >20,000/μL; with androgen therapy and during periods of observation, platelets were maintained above 5,000/μL. Patients who were refractory to platelet transfusions did not receive prophylactic infusions but could be treated with Amicar (aminocaproic acid; Lederle, Wayne, NJ). Visceral, intracranial, and severe mucocutaneous bleeding was treated with aggressive platelet transfusions in all patients. Neutropenic patients with fever were fully evaluated for an infectious source and treated with parenteral, broad-spectrum antibiotics to include a cephalosporin, amingloocyste, and semisynthetic penicillin in full doses for at least ten days; isolation and granulocyte transfusion were not routinely used. Amphotericin B was administered to patients who continued to be febrile or whose fever recurred despite antibiotic therapy.

Data Analysis

Three methods were used to assess response. First, the transfusion dependence of patients was determined by the principal investigators and entered in the data collection forms. Second, selected blood counts (hemoglobin level, reticulocyte count, absolute neutrophil count [ANC], and platelet count) and the transfusion records at 0, 3, 6, and 12 months were reviewed by two clinicians without knowledge of the patients' diagnosis or treatment. Patients were judged to have (1) normal blood counts, (2) transfusion independence with subnormal blood counts, (3) clinically significant improvement in blood counts with continued dependence on transfusion (ie, increase in ANC from below to above 500/μL, increase in platelets from <5,000/μL to >20,000/μL), (4) increased peripheral blood counts that were not clinically meaningful, (5) no improvement, or (6) died. Third, values of absolute reticulocyte count, platelet number, and ANC before and after treatment were compared. For examination of the effect of treatment on platelets and neutrophils together, a score was devised that linearly combined the final neutrophil and platelet counts (P-N score = platelet number/10^9 + neutrophil/50); this score was devised to equalize a final platelet count of 20,000/μL with a final granulocyte count of 1,000/μL (similar results were obtained with other permutations that, for example, equalized a platelet count of 20,000/μL with 500 or 1,500/μL granulocytes).

RESULTS

Severe Aplastic Anemia (Group I)

Patient characteristics. Seventy-seven patients with severe disease were randomized to receive either ten (N = 41) or 28 (N = 36) days of ATG. One patient randomized to receive 28 days of treatment in fact received treatment for only ten days; for analysis purposes, he was included in the 28-day group. Two patients who entered the protocol but died before receiving the first dose of ATG were included in the analysis. In one case, a mistaken diagnosis of aplastic anemia for hairy-cell leukemia led to randomization; this patient was not included in the analysis.

The clinical and hematologic characteristics of the two randomization arms were approximately equal (Table 1). Although there were no statistically significant differences with respect to age and sex between the two randomization groups, there were more women and older patients in the 28-day treatment arm. The time from diagnosis (determined by bone marrow biopsy) to treatment with ATG was significantly longer for the 28-day group, but the absolute difference in means was less than ten days (no attempt was made to estimate duration of disease based on onset of symptoms). The great majority of patients in both randomization arms had idiopathic disease.
Table 1. Group I Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>10 d</th>
<th>28 d</th>
<th>P</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>34 ± 23 (41)</td>
<td>43 ± 26 (36)</td>
<td>.14</td>
</tr>
<tr>
<td>Females (%)</td>
<td>56 (41)</td>
<td>61 (36)</td>
<td>.66</td>
</tr>
<tr>
<td>Time from diagnosis (d)</td>
<td>20 ± 13 (35)</td>
<td>29 ± 22 (34)</td>
<td>.04</td>
</tr>
<tr>
<td>Idiopathic etiology (%)</td>
<td>78 (40)</td>
<td>78 (36)</td>
<td>.98</td>
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<tr>
<td>Marrow cellularity (%)</td>
<td>9 ± 9 (39)</td>
<td>8 ± 6 (35)</td>
<td>.72</td>
</tr>
<tr>
<td>Hb at presentation (g/dL)</td>
<td>7.6 ± 2.3 (32)</td>
<td>8.1 ± 2.2 (29)</td>
<td>.36</td>
</tr>
<tr>
<td>ANC at presentation (N/µL)</td>
<td>572 ± 791 (26)</td>
<td>563 ± 782 (24)</td>
<td>.97</td>
</tr>
<tr>
<td>Platelets at presentation (N x 10^11/µL)</td>
<td>44 ± 161 (38)</td>
<td>24 ± 35 (32)</td>
<td>.50</td>
</tr>
<tr>
<td>RBC transfusion (%)</td>
<td>88 (41)</td>
<td>86 (36)</td>
<td>1.0</td>
</tr>
<tr>
<td>Platelet transfusion (%)</td>
<td>66 (41)</td>
<td>58 (36)</td>
<td>.50</td>
</tr>
</tbody>
</table>

NOTE. Hb, hemoglobin; ANC, absolute neutrophil count; RBC, red blood cell. RBC transfusion and platelet transfusion indicates the proportion of each population that had received these blood products prior to therapy. Sample size for each determination is shown in parentheses.

Two sets of hematologic data were collected by the principal investigators. The first represented historic values from the time of presentation, which should be unaffected by transfusion; these blood counts were usually provided by referring physicians. The second set of blood counts was obtained at the referral institution; although more uniform in quality, these values (with the exception of the absolute neutrophil number) were heavily contaminated by recent transfusions. Neither set of values was statistically different between the two groups.

Outcomes. Outcomes were assessed in three different ways. Investigators recorded the anticipated transfusion status of their patients at 3 months after initiation of ATG treatment. Transfusion independence has been employed traditionally as a measure of success in the treatment of aplastic anemia, and freedom from transfusion obviously represents a substantial gain for the patient. However, these were clearly subjective judgments in an unblinded study; in addition, transfusion status need not correlate with neutrophil values. Although blood counts have the advantage of objectivity, their use as a measure of response has several important disadvantages: (1) in nonresponding patients, hemoglobin level, absolute reticulocyte number, and platelet count are strongly affected by transfusion; (2) there is a high degree of error in the measurement of reticulocytes and of platelets at low absolute platelet values; and (3) blood counts are censored by death. As a third measure of efficacy, the abstracted clinical records, including serial blood counts and transfusion histories, were examined by two hematologists who were blinded to patients’ treatment assignments and graded on a scale devised to reflect clinically meaningful improvement.

As shown in Table 2, about one third of the patients entered in both randomization arms were transfusion independent at 3 months. Of patients surviving to 3 months, almost one half in each group were transfusion independent.

There were some differences in the blood counts between patients treated for ten days with ATG and patients treated for 28 days with ATG (Table 2). The mean ANC was about 500/NL higher in patients treated for 28 days with ATG compared with those treated for ten days with ATG, and there was a similar difference in the improvement in neutrophil values.

Table 2. Group I Outcomes at 3 Months

<table>
<thead>
<tr>
<th></th>
<th>10 d</th>
<th>28 d</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfusion independence of total</td>
<td>32 (41)</td>
<td>31 (36)</td>
<td>.98</td>
</tr>
<tr>
<td>Survivors</td>
<td>42 (31)</td>
<td>46 (25)</td>
<td>.77</td>
</tr>
<tr>
<td>Clinical status</td>
<td>(41)</td>
<td>(36)</td>
<td></td>
</tr>
<tr>
<td>Normal blood counts</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>T₃₁-independent</td>
<td>17 ± 43.9</td>
<td>17 ± 50.0</td>
<td>.59</td>
</tr>
<tr>
<td>Significant improvement</td>
<td>24</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Higher blood counts</td>
<td>10</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>22</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>24</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Blood counts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final ANC</td>
<td>1,116 ± 1,074 (29)</td>
<td>1,610 ± 1,156 (24)</td>
<td>.11</td>
</tr>
<tr>
<td>Increment in ANC</td>
<td>605 ± 1,157 (28)</td>
<td>1,100 ± 1,140 (23)</td>
<td>.13</td>
</tr>
<tr>
<td>Final absolute reticulocytes</td>
<td>10 ± 62 (9)</td>
<td>11 ± 86 (6)</td>
<td>.68</td>
</tr>
<tr>
<td>Final platelets</td>
<td>37 ± 42 (28)</td>
<td>52 ± 25 (19)</td>
<td>.27</td>
</tr>
<tr>
<td>Platelet-neutrophil score</td>
<td>58 ± 47 (26)</td>
<td>88 ± 60 (19)</td>
<td>.06</td>
</tr>
</tbody>
</table>

All values are expressed as percentages except blood counts (ANC – N/µL; platelet count – N x 10^11/µL; reticulocyte count – N x 10^11/µL). Sample size for each determination is shown in parentheses.
judged to be responders had much higher blood counts than nonresponders (Table 3). Twenty-four percent of the patients who received ATG for ten days was 70% (N = 31), while the 28-day group had a much higher death rate of 53%, compared with 19% for younger women (six of 21 or 29%) and younger men (three of 22 or 14%) (P = 0.28).

As expected, the baseline neutrophil number was a major prognostic factor. Forty-four percent of patients with neutrophil counts <200/µL (N = 36) died before 3 months, but only 11% of those with counts >200/µL (N = 37) died; severely neutropenic patients also had a much lower likelihood of achieving transfusion independence or a favorable clinical classification. However, analysis of patients who survived for 3 months showed that similar proportions of severely neutropenic and more moderately neutropenic patients had improved (for ANC <200/µL, 70% were improved) in addition, the improvement in neutrophil number in surviving patients was greater in this severely affected population (mean final ANC 1,221/µL, mean increase from baseline 1,136; N = 20) compared with patients with baseline ANC >200/µL (mean final ANC 1,457/µL, mean increase 630; N = 31).

When combined, the three variables of age, sex, and baseline neutrophils were predictive of death (the P values associated with each of these three variables was <.05 by multiple logistic regression). The ten- and 28-day treatment groups were not balanced in respect to these variables. Larger proportions of older women and severely neutropenic patients were found in the longer treatment arm. When these differences were controlled for using multiple logistic regression, the higher rate of death associated with the 28-day treatment seen with the raw data disappeared, and there was a slightly higher risk of death (not statistically significant) with the newer treatment arm. With use of the same covariates, the probability of clinically significant improvement was greater in the longer treatment group (P = 12).

It was difficult to analyze patients with presumed specific etiologies for their bone marrow failure, such as hepatitis or chemical exposure, because of sparseness in individual groups. The 17 patients with nonidiopathic disease as a group had a much higher death rate of 53%, compared with 19% for

Table 3. Hematologic Values in Responding and Nonresponding Patients (Group I)

<table>
<thead>
<tr>
<th></th>
<th>Responders</th>
<th>Nonresponders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final ANC</td>
<td>1,740 ± 1,105 (35)</td>
<td>562 ± 696 (18)</td>
</tr>
<tr>
<td>Increment in ANC</td>
<td>1,253 ± 1,201 (34)</td>
<td>-21 ± 347 (17)</td>
</tr>
<tr>
<td>Final platelets</td>
<td>56 ± 50 (32)</td>
<td>14 ± 13 (15)</td>
</tr>
<tr>
<td>Final reticulocytes</td>
<td>11 ± 7 (34)</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Platelet-neutrophil score</td>
<td>90 ± 54 (31)</td>
<td>27 ± 16 (14)</td>
</tr>
</tbody>
</table>

NOTE. Blood counts shown were obtained at 3 months post-ATG therapy. ANC expressed as N/µL; platelet count as N x 10³/µL; reticulocyte count as N x 10³/µL. Sample size for each determination is shown in parentheses.

Fig 2. Improvement in absolute neutrophil number after ATG therapy. In all figures, the abscissa shows time in days following initiation of treatment. (A) Change in ANC in all patients in group I treated with either ten or 28 days of ATG. A slope was generated for each patient using all available ANC numbers from 0 to 3 months. A t-test was used to compare values between groups, and the figure depicts the average slopes for the ten and 28-day groups. The slopes are significantly different (P = .05). (B) Histogram showing distribution of all group I patients who recovered and whose initial ANC was <200/µL against time at which a neutrophil value of 1,000/µL was achieved.

platelet counts were also slightly higher in surviving patients who received ATG for 28 days. These results suggested a trend toward higher final blood counts in the patients who received more ATG; a score that combined final neutrophil and platelet values did show a nearly statistically significant difference between ten and 28 days of treatment (P = .06). In addition, the rate of rise of neutrophil numbers in all patients who were treated with the longer course of ATG was higher than that of the ten-day group (Fig 2A).

Scoring for clinical improvement also showed comparable results for both randomization groups (Table 2). Forty-four percent of the patients who received ATG for ten days showed clinically significant improvement compared with 50% of the group treated for 28 days. As a group, patients judged to be responders had much higher blood counts than did nonresponders (Table 3). Twenty-four percent of the shorter treatment group died before 3 months vs 31% of the longer treatment group. There was a trend toward more complete improvement in patients who received more ATG, as shown by the slightly higher number of complete responders and higher final blood counts in the 28-day ATG group (Table 2).

Variables affecting outcome. For assessment of prognostic risk factors, all patients with severe acute aplastic anemia were considered as a single group. Four factors were

- Age: older patients had a higher rate of death.
- Sex: women had a higher death rate than men.
- Baseline neutrophils: patients with lower baseline neutrophil counts had a higher death rate.
- Baseline platelet counts: patients with lower baseline platelet counts had a higher death rate.

In all figures, the abscissa shows time in days following initiation of treatment. (A) Change in ANC in all patients in group I treated with either ten or 28 days of ATG. A slope was generated for each patient using all available ANC numbers from 0 to 3 months. A t-test was used to compare values between groups, and the figure depicts the average slopes for the ten and 28-day groups. The slopes are significantly different (P = .05). (B) Histogram showing distribution of all group I patients who recovered and whose initial ANC was <200/µL against time at which a neutrophil value of 1,000/µL was achieved.
transfusion dependent at 1 year. The remaining patients were evenly divided between recovery to transfusion independence and death. With the criteria of transfusion independence or meaningful clinical improvement, a plateau in recovery appeared at about 3 months (Fig 5). The use of logistic regression analysis showed that there was a strong correlation between clinical status (consensus A = 5, B = 4, C = 3, D = 2, E = 1) and vital status at 1-year (P = .01). Some improvement in blood counts measured at 3 months was a powerful predictor of 1 year survival: 27 of 30 patients with some hematologic improvement at 3 months were alive at 1 year, while only three of nine without improvement survived (P = .03 by chi-square test). Two late deaths occurred in patients who were judged to be clinically improved at 3 months.

Death. The most frequent cause of death in patients with acute severe disease was infection, and only three patients died of a bleeding complication (one of which occurred as a complication of catheter placement). In most cases of death due to an infectious cause, positive cultures were of common bacterial and fungal species.

Chronic Severe and Moderate Aplastic Anemia (Group II)

Patient characteristics. There were no statistically significant differences in clinical and hematologic variables at baseline in patients randomized to receive either ATG or androgen (Table 4). Twenty-seven patients had chronic severe disease, eight had chronic moderate disease, and six

![Fig 3. Kaplan-Meier survival curve for total group I patients.](image)

59 patients with idiopathic disease (P = .01). Surviving patients in each group had similar clinical improvement (63% significant improvement in nonidiopathic disease, 65% in idiopathic group). Of six patients with hepatitis-associated aplasia, one died before 3 months and two recovered; of seven patients with drug-associated aplasia, five died before 3 months and the remaining two recovered.

Other factors that were analyzed but not found to be predictive of mortality or recovery included time from diagnosis to treatment, macrocytosis, baseline reticulocyte count, baseline platelet count, and bone marrow cellularity.

Course over first year post-ATG. In this study, patients were followed for 1 year, with clinical and laboratory evaluations scheduled for 3, 6, and 12 months. However, there were no recommendations for other forms of therapy after 3 months.

Long-term survival was estimated at 58% by the Kaplan-Meier method (Fig. 3). One-year follow-up of acute severe aplastic anemia patients treated with ATG suggested that the failed, transfusion-dependent state was unstable (Fig 4). Although 42% of patients who entered the study were transfusion dependent at three months, only about 10% were
had acute moderate aplastic anemia. There was a somewhat higher proportion of patients with chronic disease compared with those with moderate disease in the ATG treatment arm.

Outcomes. Patients in this group were analyzed in a manner similar to that used for patients with acute severe aplastic anemia in group I (Table 5). A larger proportion of patients treated with ATG were transfusion independent at 3 months in comparison with those treated with androgens. Patients who had received ATG had higher final ANC and platelet values compared with those treated with androgens, and the combined score was significantly different (Table 5). The most striking difference between the two treatment arms was in clinically significant improvement: 28% of ATG-treated patients had responded, compared with no patients receiving high-dose androgen.

When analyzed separately, the response rate to ATG was similar in patients with chronic severe aplastic anemia (five of 17) compared with patients with chronic moderate aplastic anemia (one of four). Only one patient with acute moderate aplastic anemia recovered with ATG therapy. Patients with chronic disease had generally lower blood counts, and these patients were more numerous in the ATG randomization arm. The response rate to ATG of patients with acute aplastic anemia in group I (18 of 41 or 44%, ten-day arm), while higher, was not statistically significantly different from the rate in those patients with chronic severe disease in group II (five of 17 or 29%). A dose-response effect was suggested when patients in groups I and II were combined and analyzed. For this purpose, androgen-treated patients were considered not to have received ATG; all patients receiving ten days of treatment had a dose equivalent to 1; and patients treated for 28 days with ATG had a dose equivalent of 2. With this model, the final neutrophil number was correlated with ATG dose ($P = .03$). The relative contributions of acuteness and ATG dose were estimated by linear regression:

\[
\text{Final ANC} = 141 + .52 \times \text{(baseline ANC)} + 354 \times \text{(time from diagnosis)} + 408 \times \text{(dose)}
\]

in which time to diagnosis = 1 if acute, 0 if chronic or moderate; dose = 1 if treated for ten days with ATG, 2 if treated for 28 days. While this equation gives a general sense of the relative contributions of these variables, inferences should be drawn cautiously because the equation does not

### Table 4. Group II Patient Characteristics

<table>
<thead>
<tr>
<th>Androgen</th>
<th>ATG</th>
<th>$P$</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>39 ± 22 (19)</td>
<td>40 ± 22 (25)</td>
</tr>
<tr>
<td>Females (%)</td>
<td>63 (19)</td>
<td>48 (25)</td>
</tr>
<tr>
<td>Time from diagnosis (d)</td>
<td>951 ± 1,416 (17)</td>
<td>842 ± 1,075 (17)</td>
</tr>
<tr>
<td>Moderate disease (%)</td>
<td>47 (19)</td>
<td>32 (25)</td>
</tr>
<tr>
<td>Marrow cellularity (%)</td>
<td>13 ± 10 (19)</td>
<td>11 ± 15 (23)</td>
</tr>
<tr>
<td>Hb at presentation (g/dL)</td>
<td>7.3 ± 2.8 (15)</td>
<td>8.8 ± 2.7 (18)</td>
</tr>
<tr>
<td>ANC at presentation (N/μL)</td>
<td>352 ± 323 (10)</td>
<td>940 ± 1,110 (12)</td>
</tr>
<tr>
<td>Platelet at presentation (N × 10⁹/μL)</td>
<td>28 ± 19 (17)</td>
<td>35 ± 20 (16)</td>
</tr>
<tr>
<td>ANC at baseline (N/μL)</td>
<td>708 ± 412 (19)</td>
<td>861 ± 629 (25)</td>
</tr>
<tr>
<td>Platelets at baseline (N × 10⁹/μL)</td>
<td>18 ± 12 (19)</td>
<td>34 ± 72 (25)</td>
</tr>
<tr>
<td>Reticulocytes at baseline (N × 10⁴/μL)</td>
<td>3.3 ± 3.3 (15)</td>
<td>2.9 ± 3.8 (22)</td>
</tr>
<tr>
<td>R.B.C. transfusion (%)</td>
<td>68 (19)</td>
<td>80 (25)</td>
</tr>
<tr>
<td>Platelet transfusion (%)</td>
<td>26 (19)</td>
<td>32 (25)</td>
</tr>
</tbody>
</table>

**NOTE.** Hb, hemoglobin; ANC, absolute neutrophil count; RBC, red blood cell. RBC and platelet transfusion refers to the proportion of each population that had received these blood products prior to treatment. Sample size for each determination is shown in parentheses.

### Table 5. Group II Outcomes at 3 Months

<table>
<thead>
<tr>
<th>Androgens</th>
<th>ATG</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfusion-independent of total</td>
<td>6 (16)</td>
<td>25 (24)</td>
</tr>
<tr>
<td>Clinical status</td>
<td>19 (25)</td>
<td>19 (25)</td>
</tr>
<tr>
<td>Normal blood counts</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Tₚ-independent</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Significant improvement</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Higher blood counts</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>No change</td>
<td>74</td>
<td>64</td>
</tr>
<tr>
<td>Death</td>
<td>11</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood counts</th>
<th>Androgens</th>
<th>ATG</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final ANC</td>
<td>591 ± 522 (15)</td>
<td>887 ± 725 (22)</td>
<td>.18</td>
</tr>
<tr>
<td>Increment in ANC</td>
<td>−83 ± 390 (15)</td>
<td>103 ± 602 (22)</td>
<td>.30</td>
</tr>
<tr>
<td>Final absolute reticulocytes</td>
<td>6.1 ± 3.8 (5)</td>
<td>8.7 ± 8.0 (10)</td>
<td>.51</td>
</tr>
<tr>
<td>Final platelets</td>
<td>12 ± 11 (15)</td>
<td>41 ± 57 (21)</td>
<td>.06</td>
</tr>
<tr>
<td>Platelet–neutrophil score</td>
<td>24 ± 18 (15)</td>
<td>60 ± 65 (20)</td>
<td>.05</td>
</tr>
</tbody>
</table>

All values are expressed as percentages except blood counts (ANC = N/μL; platelet counts = N × 10⁹/μL; reticulocyte count = N × 10⁴/μL). Sample size for each determination is shown in parentheses.
represent a strict stratification analysis and was derived in
the absence of two experimental groups (no treatment in
group I, 28 days of treatment with ATG in group II).

Although designed as a crossover trial, only about one half
of the patients in group II actually received a second,
alternative form of therapy, mainly because patients were
unwiling to receive male hormones. Of 16 patients who had
failed nandroline decanoate and then received ATG, four
(25%) were transfusion independent at 3 months (after
receipt of ATG). Of eight patients who failed ATG and then
received androgen, one (13%) was judged transfusion inde-
pendent at 3 months (after androgens). Because of the
likelihood that failure to complete the protocol was not
random, these data were not subjected to statistical analysis.
A paired t-test analysis of the difference in blood counts at
the completion of the first treatment regimen compared with
blood counts at the completion of the second regimen showed
significant improvement in those patients who received ATG
after androgen therapy (Table 6); there was no such
improvement in the group of patients who received andro-
gen after ATG therapy, but the small number of patients in
this category, likely the result of selection bias, diminishes
confidence in the validity of this statistical analysis.

Other Bone Marrow Failure States (Group III)

Outcomes. This group represented a heterogeneous
collection of bone marrow failure conditions; all patients
received ATG for ten days. In this group were pancytopenia
with cellular bone marrow (eight patients), amegakaryocytic
thrombocytopenia (six patients), pure red cell aplasia (four
patients), myelofibrosis (three patients), immune panlenico-
penia (two patients), cyclic neutropenia (two patients),
paroxysmal nocturnal hemoglobinuria (one patient), Fan-
coni’s anemia (one patient), myelodysplasia (one patient),
and myelodysplasia following chemotherapy (one patient).
Only two patients with pancytopenia and cellular bone
marrow and one patient with immune panlenocopenia recov-
ered to transfusion independence at 3 months.

Toxicity of ATG

Combining the three groups, adverse reactions ascribed to
ATG were reported in 73% of cases. Fever and an urticarial
eruption on the first and second day of administration of
ATG were common. An illness compatible with serum
sickness with horse serum therapy is probably much
frequent symptoms were fever, a diffuse maculopapular
eruption, pruritus, polyarthralgia, myalgia, headache, nau-
sea, and lymphadenopathy, usually lasting for several days
with onset five to 20 days after initiation of ATG treatment.
Change in urine sediment and hepatic dysfunction in the
setting of serum sickness were each described in a patient.
Adverse reactions and serum sickness were similar in all
three groups of patients. However, this study was not
designed to assess the toxicity of ATG, and the true incidence
of serum sickness with horse serum therapy is probably much
higher.13

DISCUSSION

ATG has been shown previously to be useful in restoring
hematopoiesis in patients with aplastic anemia. This multi-
center trial has confirmed the effectiveness of a commercial
ATG preparation. In patients with acute severe aplastic
anemia, ATG therapy was associated with independence
from transfusion in about one third and clinical improvement
in one half of cases at 3 months; the larger number of patients
who showed any improvement in blood counts at 3 months,
even if not clinically meaningful, had a significantly
improved likelihood of 1-year survival compared with
patients who had no increment in peripheral blood values.
These results can be compared with those obtained in other
trials of ALG in severe acute aplastic anemia in Europe
(mainly Paris; Basel, Switzerland; and Genoa, Italy); 50%
remission rate for several different types of immunosuppres-
sion, N = 170,4 the Netherlands (35%, N = 20),14 the
United Kingdom (33%, N = 15),15 and the United States
(69% maximum response, 62% continued response, N = 29),4
and of ATG in Cleveland (54%, N = 11),16 Los Angeles
(56%, N = 41),3 Minneapolis (53%, N = 19)17 and Seattle
(44%, including minimal improvement, N = 34).18 The
response rate most likely varies with time. Some patients who
respond to ATG or ALG will later relapse, although no
relapses were documented in the current study. In addition,
improvement many months following the administration of
horse serum may occur. In the multicenter trial, some
patients appeared to improve between 3 and 6 months after
ATG treatment. Late improvement was cited in the first
report of ALG effectiveness (35% of patients becoming
self-sustaining between 3 and 9 months)19 and in other trials
as well (10% to 19%13 of the responses occurred between 3
and about 6 months). Crediting late responses to treatment
has always been problematic, but the pattern of blood count
improvement does have implications for the interpretation of
other interventions in patients “refractory” to ATG.

A major goal of this study was to address quantitatively
the effectiveness of ATG. The 95% confidence interval of the
47% overall response rate in group I patients was 36% to
58%. A future study to distinguish between true response
rates of 40% and 60% (with a power of 80%, α = .05,
two-sided) would have to be considerably larger (N ~ 300).
Can the data now available help to guide the treatment of
patients in the future? There are little laboratory data to
suggest major biochemical or immunologic differences in
ATG and ALG preparations,20 yet reported remission rates
have varied fourfold (25% to 85%). These differences are

| Table 6. Changes in Granulocyte and Platelet Number After Crossover |
|---------------------|-----|-----|-----|
|                     | Mean| SD  | N  |
| Androgen → ATG      | 8.7 | 9.9 | 14 |
| Platelet            | 444 | 730 | 13 |
| Granulocyte         | −1.8| 5.4 | 4  |
| ATG → androgen      | 135 | 990 | 4  |

The means represent the difference in mean values at the end
and beginning of the crossover to alternative therapy. P values were obtained
by a paired t-test.
probably methodologic in origin. First, the criteria for improvement are often either unspecified or vaguely defined. Survival, the simplest measure of therapeutic effectiveness, is correlated with, but not equivalent to, hematologic improvement, and it is highly dependent on supportive care. While hematologic improvement in an individual patient in usually unequivocal, developing standard criteria for hematopoietic recovery in populations is difficult. Populations of patients with aplastic anemia are clinically heterogeneous, especially when viewed over time.

A second major source of variability among clinical studies is patient selection. Relatively nonstringent criteria for admission to this multicenter trial were purposely set to approximate the real use of ATG in common clinical settings. Not surprisingly, there were a large number of early deaths, especially among elderly patients and those with severe neutropenia; in these patients, ATG had no opportunity to be effective (indeed, two deaths occurred before ATG was administered). Efficacy rates will naturally be higher in studies that eliminate such high-risk participants. In our trial, patients with nonidiopathic disease had a higher mortality rate; these are frequently the patients subjected to “observation” for spontaneous recovery. Patients with a poor prognosis may also be eliminated at some centers by waiting lists or other logistic barriers to prompt treatment.

Are there practical modifications of ATG administration that can increase its efficacy in aplastic anemia? The use of haploidentical bone marrow transplantation with ATG or ALG has not been supported by controlled studies. While the highest response rate to ALG has been reported in combination with high-dose corticosteroids and chronic androgen administration, this regimen has not been as successful elsewhere, and a controlled study failed to demonstrate the value of added male hormone treatment to ATG. Although there were few statistically significant differences between ten and 28 days of ATG treatment in patients with acute severe aplastic anemia, there was a trend toward better clinical improvement and transfusion independence and more complete recovery of blood counts in patients who received the higher dose of ATG. That a dose of ATG higher than 150 mg/kg might be desirable also has been suggested by the recovery of some patients with multiple immunosuppressive therapies, given as repeated courses of ATG or ALG or sequential combinations of ALG, high-dose corticosteroids, and cyclosporine A. However, relatively low doses of ALG also have been effective in some trials. If higher doses of ATG are administered, the horse serum should be infused over a shorter period of time than 1 month to avoid the rapid clearance of heterologous protein from the circulation that follows the development of serum sickness in almost all patients at about day ten. (At the Clinical Center of the National Institutes of Health, we currently administer ATG at 40 mg/kg/d for four days.)

The effectiveness of ATG in patients with chronic or moderate disease was tested directly in group II. Patients with a longer duration between diagnosis or onset of symptoms and treatment have been reported to do both better and worse than those with acute disease. Although we found no statistical difference in recovery between acute and chronic aplastic anemia patients treated with a comparable ten-day regimen, there was a trend toward a higher response rate and greater increases in blood counts in patients with acute disease. In the group II patients, in whom a good prognosis permitted randomization between ATG and nandrolone decanoate, ATG appeared to be superior to high-dose nandrolone decanoate. The value of androgen therapy in aplastic anemia, either alone or in combination with ATG, remains unsettled after many contradictory studies. Although occasional individual patients may show androgen-dependent improvement, controlled trials have generally failed to demonstrate a survival advantage for anabolic steroid–treated populations. Even the most enthusiastic recent reports indicate little influence of androgen therapy on the survival of patients with severe disease, and the likelihood of hematologic response has been inversely correlated with the degree of severity. In our study, the response rate to nandrolone decanoate was low, with only one patient showing a clear response during the second, alternative treatment phase of group II. About one half of the patients entered this study having failed a previous course of androgens, possibly resulting in bias against androgens. Because of the possibility of bias in selection, it is probably fairer to state explicitly that ATG is effective in patients with chronic aplastic anemia rather than superior to androgen therapy.

ATG also appeared to be effective in some patients with pancytopenia and a cellular bone marrow, either because of difficulty in differentiating hypoplastic from normally cellular bone marrow biopsies or because of a similar underlying pathogenic mechanism in two distinct syndromes. A patient with probable isolated leukopenia also recovered, similar to another case in the literature treated with ATG. Too few patients with other bone marrow failure syndromes were treated to dismiss ATG as a possible effective therapy.

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The Upjohn Company provided antithymocyte globulin for the patients in this trial and administrative grants to the institutions; Dr William Finch, Dr Carl Lewis, Jeff Swartz, and James Wunderly of Upjohn assisted in the organization of the study and data collection. Dr Margaret Wu of the National Heart, Lung, and Blood Institute helped in the statistical design. Dr Eileen Leonard of the National Institutes of Health participated in the assessment of patients’ responses. Dr Geraldine Schecter of the Veterans Administration Hospital, Washington, DC; Dr Daniel Rosenbloom; and Dr Harry Keiser of the National Heart, Lung, and Blood Institute constituted the oversight committee.

REFERENCES


3. Speck B, Gratwohl A, Nissen C, Leibundgut, Ruggero D,


A multicenter trial of antithymocyte globulin in aplastic anemia and related diseases

N Young, P Griffith, E Brittain, G Elfenbein, F Gardner, A Huang, D Harmon, J Hewlett, J Fay and K Mangan