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 nandrolone decanoate. 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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Blood
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ATG). All Group III patients received ATG for ten days.

into the protocol, and 19 had never been treated with androgens; 23

of severe disease and a correspondingly

neutropenia and severe aplastic anemia

hemoglobinuria.

Twenty-two patients from groups I and II, treated at a single

center, have been reported separately.15

Protocol Outline and Randomization

Randomization was performed separately for each institution in a

block size 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 effective-

ness 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 ran-

domized to receive either ATG for ten days or nandrolone decanoate

5 mg/kg/d 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 warr-

anted exclusion from treatment. Methylprednisolone was adminis-

trated 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 institu-
tions. During periods of ATG infusion, platelet counts were main-
tained 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 prophyl-
ic infusions but could be treated with Amicar (aminocaproic acid;

Lederle, Wayne, NJ). Visceral, intracranial, and severe mucocu-

taneous 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, aminoglycoside, and semisyn-

thetic 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 subnor-

mal 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/10³ + 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>
</tr>
<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 10³/µL)</td>
<td>44 ± 161 (38)</td>
<td>24 ± 35 (32)</td>
<td>.50</td>
</tr>
<tr>
<td>ANC at baseline (N/µL)</td>
<td>466 ± 524 (39)</td>
<td>420 ± 648 (34)</td>
<td>.74</td>
</tr>
<tr>
<td>Platelets at baseline (N x 10³/µL)</td>
<td>64 ± 163 (41)</td>
<td>29 ± 43 (36)</td>
<td>.22</td>
</tr>
<tr>
<td>Reticulocytes at baseline (N x 10⁴/µL)</td>
<td>2.5 ± 2.6 (34)</td>
<td>1.5 ± 2.1 (31)</td>
<td>.09</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 ANG was about 500/µL 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 neutro-
Judged to be responders had much higher blood counts than YOUNG ET AL. 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 = .06). (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.

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 = .06). (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.

Scoring for clinical improvement also showed comparable results for both randomization groups (Table 2). 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 judged to be significantly improved; for ANC >200/µL, 64% 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 1,213; 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 ten-day ATG 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...
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.

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

![Fig 4. Proportions of patients in group I who were transfusion dependent, transfusion independent (as determined by principal investigators), and dead prior to treatment and at 0, 3, 6, and 12 months post-ATG. ](image)

![Fig 5. Meaningful hematologic improvement (A) and independence from transfusion (B), both determined by consensus, during first year following ATG therapy. Some patients were lost to follow-up after 3 months: the number assessed was 77 at 3 months, 72 at 6 months, and 60 at 1 year. ](image)
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:

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

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></th>
<th>Androgen</th>
<th>ATG</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>39 ± 22 (19)</td>
<td>40 ± 22 (25)</td>
<td>.83</td>
</tr>
<tr>
<td>Females (%)</td>
<td>63 (19)</td>
<td>48 (25)</td>
<td>.32</td>
</tr>
<tr>
<td>Time from diagnosis (d)</td>
<td>951 ± 1,416 (17)</td>
<td>842 ± 1,075 (17)</td>
<td>.81</td>
</tr>
<tr>
<td>Moderate disease (%)</td>
<td>47 (19)</td>
<td>32 (25)</td>
<td>.30</td>
</tr>
<tr>
<td>Marrow cellularity (%)</td>
<td>13 ± 10 (19)</td>
<td>11 ± 15 (23)</td>
<td>.68</td>
</tr>
<tr>
<td>Hb at presentation (g/dL)</td>
<td>7.3 ± 2.8 (15)</td>
<td>8.8 ± 2.7 (18)</td>
<td>.13</td>
</tr>
<tr>
<td>ANC at presentation (N/μL)</td>
<td>352 ± 323 (10)</td>
<td>940 ± 1,110 (12)</td>
<td>.12</td>
</tr>
<tr>
<td>Platelets at presentation (N × 10⁴/μL)</td>
<td>28 ± 19 (17)</td>
<td>35 ± 20 (16)</td>
<td>.30</td>
</tr>
<tr>
<td>ANC at baseline (N/μL)</td>
<td>708 ± 412 (19)</td>
<td>861 ± 629 (25)</td>
<td>.36</td>
</tr>
<tr>
<td>Platelets at baseline (N × 10⁴/μL)</td>
<td>18 ± 12 (19)</td>
<td>34 ± 72 (25)</td>
<td>.34</td>
</tr>
<tr>
<td>Reticulocytes at baseline (N x 10⁴/μL)</td>
<td>3.3 ± 3.3 (15)</td>
<td>2.9 ± 3.8 (22)</td>
<td>.69</td>
</tr>
<tr>
<td>R.B.C. transfusion (%)</td>
<td>68 (19)</td>
<td>80 (25)</td>
<td>.49</td>
</tr>
<tr>
<td>Platelet transfusion (%)</td>
<td>26 (19)</td>
<td>32 (25)</td>
<td>.68</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>
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<th>Androgen</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>
<td>.21</td>
</tr>
<tr>
<td>Clinical status</td>
<td>(19)</td>
<td>(25)</td>
<td></td>
</tr>
<tr>
<td>Normal blood counts</td>
<td>0</td>
<td>4</td>
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</tr>
<tr>
<td>$T_r$-independent</td>
<td>0</td>
<td>8</td>
<td>.28</td>
</tr>
<tr>
<td>Significant improvement</td>
<td>0</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Higher blood counts</td>
<td>16</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>74</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>11</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Blood counts</td>
<td></td>
<td></td>
<td></td>
</tr>
<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 × 10⁴/μL; reticulocyte count = N × 10⁴/μL). Sample size for each determination is shown in parentheses.
MULTICENTER TRIAL OF ATG

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 unwilling to receive male hormones. Of 16 patients who had failed nandrolone decanoate and then received ATG, four (25%) were transfusion independent at 3 months (after receiving ATG). Of eight patients who failed ATG and then received androgen, one (13%) was judged transfusion independent 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 androgens 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 panleukopenia (two patients), cyclic neutropenia (two patients), paroxysmal nocturnal hemoglobinuria (one patient), Fanconi’s anemia (one patient), meyelodysplasia (one patient), and meyelodysplasia following chemotherapy (one patient). Only two patients with pancytopenia and cellular bone marrow and one patient with immune panleukopenia recovered 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 urticular eruption on the first and second day of administration of ATG were common. An illness compatible with serum sickness was described in 47% of all patients: the most frequent symptoms were fever, a diffuse maculopapular eruption, pruritus, polyarthralgia, myalgia, headache, nausea, and lymphadenopathy, usually lasting for several days with onset five to 20 days after initiation of ATG treatment. Changes 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.

DISCUSSION

ATG has been shown previously to be useful in restoring hematopoiesis in patients with aplastic anemia. This multicenter 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 immunosuppression, N = 170), The Netherlands (35%, N = 20), the United Kingdom (33%, N = 15), and the United States (69% maximum response, 62% continued response, N = 29), and of ATG in Cleveland (54%, N = 11). Los Angeles (56%, N = 41), Minneapolis (53%, N = 19) and Seattle (44%, including minimal improvement, N = 34). 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) and in other trials as well (10% to 19% 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%, a two-sided test) 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, yet reported remission rates have varied fourfold (25% to 85%). These differences are

Table 6. Changes in Granulocyte and Platelet Number After Crossover

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>N</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgen → ATG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet</td>
<td>8.7</td>
<td>9.9</td>
<td>14</td>
<td>.006</td>
</tr>
<tr>
<td>Granulocyte</td>
<td>444</td>
<td>730</td>
<td>13</td>
<td>.05</td>
</tr>
<tr>
<td>ATG → androgen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet</td>
<td>-1.8</td>
<td>5.4</td>
<td>5</td>
<td>.50</td>
</tr>
<tr>
<td>Granulocyte</td>
<td>-135</td>
<td>990</td>
<td>4</td>
<td>.80</td>
</tr>
</tbody>
</table>

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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