Antithymocyte Globulin and Cyclosporine for Severe Aplastic Anemia
Association Between Hematologic Response and Long-term Outcome

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In patients with aplastic anemia, bone marrow failure leads to pan-cytopenia; death occurs secondary to infection, bleeding, or complications of severe anemia.1 Clinical observations and laboratory experiments have implicated an immune pathophysiological effect, in which T cells destroy hematopoietic stem and progenitor cells.2 Allogeneic bone marrow transplantation replaces stem cells. Current transplant methods produce high cure rates, but only a minority of patients have histocompatible siblings, and graft vs host disease is a frequent complication in older recipients.3

Observation of recovery despite failed bone marrow transplant led to the treatment of aplastic anemia with immunosuppressive therapy alone. Antithymocyte globulin improved blood cell counts in about half of treated patients.4,5 Cyclosporine improved outcomes of many who had not responded to antithymocyte globulin.6,7 The combination of antithymocyte globulin, which lysed lymphocytes, and cyclosporine, which blocks T-cell function, has led to survival rates comparable with those observed with transplant recipients.8-10

However, immunosuppression is an imperfect treatment. About a third of patients fail to respond, and even responders often have chronically low blood cell counts. Late complications include relapse of pancytopenia and development of secondary clonal hematologic diseases like myelodysplasia.11 These problems have resulted in pessimistic commentary suggesting that immunosuppression only "postpones the inevitable"12 use of more toxic therapies such as high-dose cyclophosphamide13 and uncertainty in the timing of risky alternative donor transplants in

Context In most patients, aplastic anemia results from T-cell–mediated immune destruction of bone marrow. Aplastic anemia can be effectively treated by stem cell transplantation or immunosuppression.

Objective To assess long-term outcomes after immunosuppressive therapy.

Design, Setting, and Patients Cohort of 122 patients (31 were ≤18 years and 91 were >18 years) with severe aplastic anemia, as determined by bone marrow cellularity and blood cell count criteria, were enrolled in a single-arm interventional research protocol from 1991 to 1998 at a federal government research hospital.

Interventions A dose of 40 mg/kg per day of antithymocyte globulin administered for 4 days, 10 to 12 mg/kg per day of cyclosporine for 6 months (adjusted for blood levels), and a short course of corticosteroids (1 mg/d of methylprednisolone for about 2 weeks).

Main Outcome Measures Survival, improvement of pancytopenia and transfusion-independence, relapse, and evolution to other hematologic diseases.

Results Response rates were 60% at 3 months after initiation of treatment, 61% at 6 months, and 58% at 1 year. The blood cell counts of patients who responded no longer satisfied severity criteria and they were transfusion-independent. Overall actuarial survival at 7 years was 55%. Survival was associated with early satisfaction of response criteria (86% vs 40% at 5 years; P < .001) and by blood counts at 3 months (reticulocyte count or platelet count of > 50 x 10^3/µL predicted survival at 5 years of 90% [64/71] vs 42% [12/34] for patients with less robust recovery [P < .001 by log-rank test]). There were no deaths among responders more than 3 years after treatment. Relapse was common, but severe pancytopenia usually did not recur. Relapse did not influence survival. Thirteen patients showed evolution to other hematologic diseases, including monosomy 7.

Conclusions Approximately half of patients with severe aplastic anemia treated with antithymocyte globulin and cyclosporine have durable recovery and excellent long-term survival. These outcomes were related to the quality of hematologic recovery.
We analyzed long-term outcomes of one of the initial protocols establishing the use of combined immunosuppressive therapy in aplastic anemia to determine the association between relapse and survival, and to determine the rate and significance of evolutionary events.

METHODS

Patients referred to the Warren Grant Magnusson Clinical Center with a diagnosis of severe aplastic anemia between December 1989 and January 1998 were evaluated for entry into the protocol. Referrals were from hematologists in private practice or at academic institutions throughout the United States and world. Data were collected until February 2002 and censored from the date of last contact with the patient.

Study inclusion required bone marrow cellularity of less than 30% and depression of at least 2 of 3 hematopoietic lineages: absolute neutrophil count of 500/µL or less, absolute reticulocyte count of 40 × 10^9/µL or less (≤60 × 10^9/µL after January 1993 to reflect changes in instrumentation), and platelet count of 20 × 10^9/µL or less. Exclusion criteria included creatinine level higher than 2 mg/dL (176.8 µmol/L), concurrent malignancy, recent radiation or chemotherapy, and poor expectation of immediate survival. The protocol was approved by the National Heart, Lung, and Blood Institute’s institutional review board and all patients or their parents gave informed consent.

Patients were treated at the Clinical Center. Immediate hypersensitivity to antithymocyte globulin was assessed by skin test; individuals with a positive reaction underwent desensitization. A dose of 40 mg/kg per day of equine antithymocyte globulin (Upjohn, Kalamazoo, Mich) was administered intravenously on days 1 through 4. A dose of 1 mg/kg per day of methylprednisolone was given concurrently to prevent or ameliorate serum sickness, usually for about 2 weeks. The initial dose of cyclosporine was 12 mg/kg per day in adults and 15 mg/kg per day in children aged 3 to 18 years, and adjusted to maintain serum levels of 200 to 400 ng/mL or for renal toxicity.

Supportive care was provided as previously described. After September 1991, short courses of granulocyte colony-stimulating factor administration. We previously showed that this definition applied in at least 7 g/dL (≥9 g/dL for patients with underlying cardiopulmonary disease). Platelets were transfused prophylactically for blood levels lower than 10 × 10^9/µL and at higher levels in the setting of clinically significant or symptomatic bleeding. Aerosolized pentamidine was administered as prophylaxis against Pneumocystis carinii during cyclosporine treatment.

Bone marrow biopsy and aspiration, including cytogenetic study, were performed before enrollment. Children and young adults had a chromosome assay to rule out Fanconi anemia, and all were tested for paroxysmal nocturnal hemoglobinuria (PNH) by the Ham test. Patients were hospitalized for the administration of antithymocyte globulin and discharged when clinically stable—usually at about 2 weeks. After discharge, patients returned to the care of their physicians for laboratory tests weekly for the first month and biweekly thereafter. Cyclosporine levels were measured every 2 weeks. Patients returned to the Clinical Center for complete evaluation at 3 and 6 months and 1 year after treatment and then annually. Bone marrow was examined morphologically and cytogenetically at 6 months, 1 year, and then annually.

Response was defined as no longer meeting blood cell count criteria for protocol inclusion in the absence of recent transfusions and granulocyte colony-stimulating factor administration. We previously showed that this definition correlated strongly with both independence from transfusion and survival at 1 year. Relapse was not defined by blood cell counts but rather as any reinstitution of immunosuppressive therapy.

Binary group comparisons were made using the chi² or Fisher exact test as appropriate. Nonbinary group comparisons were made with the Kendall statistic. Survival curves were calculated using the Kaplan-Meier method and compared using the log-rank test. Cumulative incidence curves were calculated as described. Cox regression was used to estimate hazard ratios for both time-fixed and time-varying covariates. All significance tests were 2-sided. Calculations were performed using Mathematica (Wolfram Research, Champaign, Ill) and SPLUS (Mathsoft, Seattle, Wash) statistical software packages.

RESULTS

Patients

We enrolled 122 patients. The ratio of male to female patients was 1.14:1 and the median age 35.0 years. Thirty-one of the patients were children or adolescents younger than age 18 years—18 were younger than 12 years and 4 were younger than 5 years. The absolute neutrophil count was less than 200/µL at presentation in 48 (39%) and less than 500/µL in 80 (66%) patients. In two thirds of cases, there was no apparent precipitating event. Onset was associated with medications in 12 patients (10%), chemicals in 10 (8%), posthepatitis in 12 (10%), Epstein-Barr virus infection in 1 (1%), and a complication of pregnancy in 3 (3%). The median time to treatment after diagnosis was 31 days. Median follow-up at the time of this analysis was 7.2 years.

Hematologic Response

Response rates were 60% at 3 months after initiation of treatment, 61% at 6 months, and 58% at 1 year. Five patients, all of whom had some early blood cell count improvement, did not meet response criteria at 3 months but were transfusion-independent and classified as responders by 6 months. Four patients were transient responders at 3 months and no longer satisfied response criteria by 6 months. On uni-
variate analysis, the likelihood of response was not associated with sex, delay between diagnosis and protocol entry, or pretreatment absolute neutrophil count. However, younger age was associated with a higher response rate ($P = .04$ with age treated as a continuous variable). Virtually all patients who were classified as responders were transfusion-independent.

**Survival and Mortality**
Overall survival of all patients at 7 years posttreatment was 55% (FIGURE 1). Patients younger than 50 years had better survival than those older than 50 years (66% vs 38%; $P = .01$). Patients with absolute neutrophil counts of less than 200/µL at presentation had worse survival than those with higher absolute neutrophil counts (48% vs 58%; $P = .02$). Excess deaths in severely neutropenic patients occurred mainly in the first few months after diagnosis. There were 12 deaths in 48 patients with severe neutropenia within 3 months of treatment compared with 3 deaths in 74 patients without severe neutropenia. Sex, ethnicity, etiology, and duration of disease were not correlated with survival.

Posttreatment status was strongly associated with long-term prognosis, as defined by response criteria (blood cell counts inconsistent with severe aplastic anemia, transfusion-independence) or by blood cell counts. For patients classified as responders at 3 months, actuarial survival at 5 years was 86% compared with 40% for nonresponders ($P < .001$; Figure 1B). For patients surviving to 3 months, the me-

**Figure 1. Survival for Entire Cohort**

A, Overall survival of 122 patients with severe aplastic anemia treated with antithymocyte globulin and cyclosporine; median follow-up was 7.2 years. B, Recovery defined by blood cell counts no longer satisfying severity criteria. C, Blood cell count values. D, Blood cell count values in patients classified as responders.
median platelet and reticulocyte counts were each approximately $50 \times 10^3/\mu L$. Survival of patients who had either or both counts above the median value was 90% (64/71 alive) at 5 years compared with 42% (12/34 alive) for patients with both counts below the median (Figure 1C). There were no deaths from disease more than 3 years after treatment among patients classified as responders at 3 months (Figure 1B). For patients who responded qualitatively, quantitative counts were still of prognostic importance. Survival for responders with more robust blood cell count recovery was 91% at 5 years compared with 47% for responders with both counts below the median ($P < .001$; Figure 1D).

Sixteen patients (13%) died before their 3-month evaluation (mostly due to fungal infection). Despite persistent thrombocytopenia, few patients died of hemorrhage. Six patients died due to complications following bone marrow transplant (immunosuppression failed in these patients). A total of 7 patients who entered the study ultimately underwent bone marrow transplantation (all from matched, unrelated donors): 4 were nonresponders and died due to transplant complications; 3 were patients classified as responders at 3 or 6 months who later evolved to myelodysplasia. Only 1 responder who relapsed with a new finding of monosomy 7 survived after a matched unrelated transplant.

**Relapse**

The requirement for further immunosuppressive therapy not specified by the protocol was common, occurring in 26 of 74 patients classified as responders at 3 months. The cumulative incidence of relapse among responders was approximately 35% at 5 years (FIGURE 2A). Age, sex, delay between diagnosis and protocol entry, absolute neutrophil count prior to treatment, or presumed etiology were not associated with relapse. Qualitative blood cell counts at 3 months were not associated with relapse.

The impact of relapse on survival was assessed using a Cox regression model with a time-varying covariate. At the point of relapse, a patient was assumed to be at a fixed increased risk of death. All 3-month responders were included in this analysis and relapse was not statistically significantly associated with death ($P > .10$).

Our relapse definition was broad because many patients restarted cyclosporine, but were only experiencing a modest decline in 1 or more blood counts. Recurrent frank severe pancytopenia was unusual, and only a minority of patients required retreatment with antithymocyte globulin. Of a total of 25 relapsing patients, 8 showed only declining blood cell counts and a further 6 again required transfusions; only 1 patient failed to respond to reinstitution (or continuation in 1 case) of cyclosporine, and none died. For 9 patients who had recurrent severe pancytopenia, 6 were retreated with antithymocyte globulin and 3 died.

While some relapsed patients died of the complications of severe pancytopenia, most responded to the reintroduction of immunosuppressive therapy. However, the majority of relapsed patients required continued administration of cyclosporine after retreatment, usually at a low dose to maintain blood cell counts above levels required to avoid transfusion. About 90% of relapsing patients continued with cyclosporine therapy for 1 to 2 years after its reinstitution and about 60% were cyclosporine-dependent at 6 to 7 years.
New Hematologic Diagnoses

Thirteen patients were considered to have evolved to a new hematologic diagnosis (Figure 2B). Cytogenetic abnormalities predominantly involved chromosome 7 (monosomy in 9 patients and deletion 7p in 1 patient) and chromosome 8 (trisomy in 2 patients). Monosomy 7 usually occurred with either a minimal initial clinical response or clinical relapse to severe pancytopenia. Seven patients with this finding died, 4 of refractory pancytopenia and 3 after evolution to acute myelogenous leukemia. In contrast, trisomy 8 was associated with an initial hematologic response followed by dependence of blood cell counts on continued cyclosporine administration, and a good long-term prognosis.

In both univariate and multivariate analysis of baseline factors, only age was associated with evolution to a new hematologic diagnosis. Each decade of life increased the risk of evolution by about 40% (P < .05). Overall, qualitative blood cell counts at 3 months were not predictive of evolution using the simple risk stratification model (both platelet and reticulocytes at 3 months < 50 × 10⁹/µL), but evolution to monosomy 7 and its poor consequences were predicted by 3-month platelet count alone (3.5-fold increased risk for patients with platelets < 50 × 10⁹/µL; P = .02).

Survival after evolution was poor, with more than 60% of evolving patients—all with monosomy 7—dead within 3 years of the event. When we assessed the impact of evolution on survival using a Cox regression model with a time-varying covariate, in which a patient was assumed to be at a different and fixed risk of death at the point of evolution, evolved patients were at 7 times the risk of death compared with those who had not evolved (P < .001).

Paroxysmal nocturnal hemoglobinuria (defined by a positive Ham test result) developed in 7 patients within 6 months of diagnosis (in all but 1 case). Early onset was reflected in event curves, in which the risk of PNH was 10% at 2 years and remained stable for at least 7 years (data not shown). More extensive analysis of PNH was not undertaken because the Ham test was supplanted by flow cytometric methods as this study was ending.

COMMENT

Historically, patients with severe aplastic anemia had a poor prognosis. In the early 1970s, 80% to 90% of patients died of complications of pancytopenia before 12 to 18 months had elapsed.¹⁸,¹⁹ The introduction first of bone marrow transplant and later of antithymocyte globulin regimens dramatically altered the clinical course of aplastic anemia. Seventy-five percent of European patients undergoing either treatment in the 1990s could expect a 5-year survival.²⁰ Antithymocyte globulin combined with cyclosporine has been especially effective in children,²¹ older adults who do not fare well with transplant,²² and in patients with severe neutropenia.²³ Despite evident short-term efficacy, questions remain about the long-term benefits of immunosuppression. Concerns have focused on reported high rates of relapse, evolution to PNH and myelodysplasia, and leukemic transformation. This study provides data about the long-term outcomes after treatment of aplastic anemia with immunosuppression in a consistently treated population, and the consequences to the patient with incomplete blood cell count responses to the combined therapy of antithymocyte globulin and cyclosporine.

We defined relapse as the requirement for further immunosuppressive therapy not specified by protocol on the conservative assumption that even modestly declining blood cell counts presaged severe pancytopenia, but the number of patients who developed frankly recurrent aplastic anemia was small. In other studies, relapse has been variably defined. In a retrospective European analysis, the actuarial risk of relapse (defined as a new requirement for transfusion) was 35% at 10 years.²⁴ In more recent studies of antithymocyte globulin combined with cyclosporine, the relapse rate was 9%, but the majority of responding patients required continued cyclosporine therapy.²⁵ These high relapse rates suggest that a large proportion of patients may still be inadequately treated by combined immunosuppressive therapy. However, relapse of aplastic anemia does not carry the same dire prognosis as relapse of leukemia. Patients who experienced relapse usually responded to reintroduction of immunosuppressive therapy, and we were unable to demonstrate an effect of relapse on survival. Lack of association between relapse and initial or recovery blood cell counts and occurrence of relapse after full hematologic recovery further suggest a mechanism of persistent or resurgent pathological immunity rather than stem cell exhaustion. Laboratory studies have shown that molecularly defined inhibitory T-cell clones are reduced but often persistent at low levels—even in recovered patients.²⁶,²⁷

Several hematologic diseases of stem cell clonal origin are associated with aplastic anemia. Most prominent is PNH, which for many years was falsely considered a common late development in treated patients, with rates of evolution cited to be as high as 25%.²⁷ However, flow cytometric assays have allowed the identification of an abnormally expanded clonal population of PNH cells in a large proportion of aplastic anemia patients at presentation, and the development of a new clone after treatment is unusual.²⁸ The second major clonal process, myelodysplasia, can be difficult to distinguish clinically from aplastic anemia and may even respond to immunosuppressive therapy.²⁹ Myelodysplasia, which is recognizable by a characteristic bone marrow morphology or by the detection of typical cytogenetic abnormalities, appears to be a true late complication of treated aplastic anemia.

Clonal evolution after aplasia occurs in stereotypical patterns—most frequently involving chromosomes 7 or 8. These aberrations have different clinical consequences.³⁰ Monosomy 7 appeared as a new event, sometimes after multiple normal cytogenetic ex-
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Auctions and was almost always associated with refractory relapse of pan-cytopenia or malignant transformation. In contrast, trisomy 8 was relatively benign and consistent with hematologic response, cyclosporine-dependence, and a stable clinical course.

Unexpectedly in this study, outcome was related to the presence of early recovery and to the quality of the blood cell count response at 3 months after receiving antithymocyte globulin. The flatness of the survival curve suggests that a significant proportion, perhaps 25% of treated patients and 50% of responding patients, are likely to be functionally cured and should not experience major complications from aplastic anemia. For this identifiable group, immunosuppression does more than postpone what was once considered inevitable. These patients also appear to have the durable improvements credited to the more toxic use of high-dose cyclophosphamide. Conversely, patients with suboptimal hematologic recovery, even if transfusion-independent, remain at risk. How best to manage the patients refractory to a single course of antithymocyte globulin remains uncertain. Options include further immunosuppression and stem cell transplant from donors other than histocompatible siblings, which, while often successful in children, continues to carry significant risk of procedure-related mortality.

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