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Therapy of Severe Aplastic Anemia with Anti-human Thymocyte Globulin (ATGAM®) with and without HLA-Haploidentical Bone-Marrow Infusion

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Six patients with severe aplastic anemia treated with horse anti-human thymocyte globulin (ATG) and androgen. Four of these patients were only given ATG (ATGAM®), 16 mg/Kg/dose x 10 doses. The remaining two cases received an infusion of maternal HLA-haploidentical marrow cells following ATG therapy. One patient had a complete response, three had a partial response, one showed minimal improvement and two were non-responders. The two patients who received the additional haploidentical marrow cells showed a hematologic recovery sooner than the ATG alone cases. The toxicity of the ATG therapy was tolerable. Long term follow up of there patients and further studies of this treatment in aplastic anemia with pediatric age group are under way.

Key Words: *Severe aplastic anemia, Anti-human thymocyte globulin (ATG, ATGAM®), HLA-haploidentical bone-marrow Infusion*

INTRODUCTION

Aplastic anemia is characterized by peripheral blood pancytopenia and bone marrow hypocellularity.

Duration of survival varies inversely with the severerity of hematologic depression at diagnosis.

Despite improvements in supportive care, less than 25% of patients with severe marrow aplasia survive. Although anabolic hormons (androgen) may benefit individual patients, use of these drugs has not improve prognosis in large groups of patients with severe aplastic anemia.

There is increasing evidence that bone marrow damage in aplastic anemia can be initiated or perpetuated by immunologic mechanisms. These data resulted in trials of immunosuppressive therapy for marrow aplasia.

Immunosuppressive therapy for severe aplastic anemia has been reported to result in hematologic improvement in 30%-60% of treated patients. In 1970 and 1972, Mathe et al. reported that antihuman lymphocyte globulin (ALG) used as a conditioning procedure before transplantation of non-HLA-identical bone marrow from related donors, had a beneficial effect in about a third of patients with severe aplastic anemia. In 1981, Speck et al., suggested that mismatched-marrow infusion following ALG enhanced the degree of hematologic recovery. The current study (Doney et al., 1984) which was designed to treat patients with severe aplastic anemia with a course of antihuman thymocyte globulin (ATG) and androgen, for those patients who had HLA-haploidentical donors, marrow cells were also infused, has reported an increased incidence of hematologic recovery.

This study was designed to treat six patients with severe aplastic anemia with a course of ATG and androgens. For two of those patients, HLA-haploidentical marrow cells were also infused.

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MATERIALS AND METHODS

Patient selection: Six patient with severe aplastic anemia who failed response to anabolic androgen (oxymetholone) therapy and were unable to obtain HLA-

Table 1. Clinical details of patients on admission

Case No.	Patients	Age (yr)	Sex	Etiology	Disease duration (mo)	Previous therapy		Therapy
						Androgen	Prednisolone	
1	S.M. Ahn	7	F	unknown	69	+	+	ATG*
2	Y.S. Choi	4	M	unknown	6	+	+	ATG+BMT**
3	H.U. Kim	5	M	drug	6	+	+	ATG
4	J.Y. Choi	10	F	unknown	5	+	+	ATG
5	J.S. Lee	7	F	unknown	45	+	+	ATG+BMT
6	M.Y. Keun	10	F	drug	10	+	+	ATG

* Anti-human thymocyte globulin
**Bone marrow transplantation

matched sibling were eligible for this study.

Clinical details of six patients on admission are shown in Table 1. Severe aplastic anemia was defined as a markedly hypoplastic marrow ($<25\%$ of normal cellularity) and at least two of following three peripheral blood criteria: granulocytes $500/mm^3$, plastelets $20,000/mm^3$, corrected reticulocytes $<1\%$. Pretreatment blood counts and bone marrow cellularities are shown in Table 2.

Before ATG therapy, patient were observed for one week to establish baseline blood counts, blood chemistry and to complete histocompatibility studies.

During this interval, with other underlying illnesses such as liver disease renal dysfunction etc. were excluded

Methods: Informed consent was obtained from the patient's parent or appropriate family members prior to ATG therapy.

Two patients were assigned to the ATG treatment with androgen and HLA-haploidentical bone marrow infusion.

Patients in all groups received standard supportive care, including platelet and-red blood cell transfusions and institution of intravenous antibiotics when fever persisted and granulocyte counts were less than $500/mm^3$.

In general, the hematocrit was maintained between 25% and 30% and the platelet count at $>20,000/mm^3$.

Anti-human thymocyte globulin (ATG, ATGAM®, Lot 17921(−5) was supplied by Upjohn company, Korean division.

All patient had negative intradermal test to a 1:1000 dilution of horse ATG.

Patients then received ATG, 16 mg/Kg/day intravenously for 10 days. Each daily dose was diluted in 500ml of half-normal saline and infused over 6-12 hours.

Premedications for the ATG infusions included diphenhydramine, acetaminophen and phenobarbital. Severe reactions such as continuous high fever, pruritic skin eruptions and arthralgias were treated with minimal doses of prednisolone, 0.5-1 mg/Kg/day. Forty-eight hours after completing ATG therapy, all patients were given oxymetholone, 3 mg/Kg P.O. daily and two patients received marrow infusion from family members with whom they were identical for one HLA-haplotype.

Table 2. Pretreatment Blood Counts

Case No.	Hb (g/dl)	Hct (%)	Corrected Reticulocyte (%)	Total WBC ($x10^3/mm^3$)	Granulocytes ($x10^3/mm^3$)	Platelets ($x10^3/mm^3$)	Marrow Cellularity (%)
1	5.4	15.8	0.5	2.3	0.23	2.3	$<20\%$
2	6.5	18.5	0.4	3.9	0.32	1.4	$<20\%$
3	7.7	22.5	0.3	3.7	0.44	0.5	$<20\%$
4	6.4	19.0	0.3	3.5	0.42	1.3	$<20\%$
5	4.2	12.8	0.7	3.6	0.61	2.6	$<20\%$
6	6.1	19.4	0.3	1.9	0.40	0.7	$<20\%$

Donors and recipients were ABO-compatible and lymphocytotoxic crossmatches were negative.

Degree of response: The subsequent sustained level of improvement were as follows: (1) Complete response - return of a normal hemoglobin and hematocrit, granulocyte count $\geq$1,000/mm^3 and a platelet count of $\geq$100,000/mm^3 (2) partial response-improvement in all three cell lines; no transfusion requirement, absence of infections and granulocyte count $\geq$500/mm^3 (3) minimal improvement-transfusion still required but increased in granulocyte count by $\geq$500/m^3, and (4) no improvement-patient remained severly aplastic at 3 months or more.

RESULTS

Toxicity: In this study all patient developed a certain degree of toxicity; fever, rash and/or arthralgia, headache etc. One patient received less than 10 prescribed doses (seven doses) because of severe serum sickness like symptoms which did not response to steroid therapy. All patient developed thrombocytopenia during the ATG infusion but only three patients required a platelet transfusion.

Hematologic response: All six patients were evaluated for response to therapy. Follow-up ranged from 4 to 12 months.

A summary of the treatment and its outcome is shown in Table 3. According to the previously listed criteria, one patient had a complete response (++), three had a partial response (+), one showed a minimal improvement (±), and two were nonresponders (−).

Two cases who received the additional maternal haploidentical bone marrow infusion were shown to increase the hematologic recovery sooner than ATG alone cases. These two patients are all transfusion-independent, with platelet count between 76×10^3 and 54×10^3/mm^3 and have>1,000 granulocytes/mm^3.

Etiology of the marrow aplasia was not considered in the analysis of responsiveness to AGT therapy because of the small number of patients with known causes. Of the two patient with drug-induced aplasia, one had a minimal improvement and another one was a non-responder.

DISCUSSION

Severe aplastic anemia, whether idiopathic or induced by known toxins, is associated with grave prognosis. Conventional therapy for aplastic anemia has included steroids, androgens and intensive supportive care with transfusions and broad spectrum antibiotics. Severe aplastic anemia has in part been treated with conventional therapy, but only 25% survive one year.

More recently, as noted above, transplantation of marrow from HLA matched siblings has restored hematologic function in patient with severe aplastic anemia and increased survival to 70% (Storb et al., 1980). While marrow transplantation has a proven advantage for those patients with a matched donor, many patients have no donor.

For this latter group attention is being directed toward the role of high dose immunosuppressive therapy.

The role of immunosuppressive therapy in aplastic anemia has been examined by numerous investigators (Mathe et al., 1970; Speck et al., 1981; Champlin et al., 1983; Doney et al., 1981 and 1984).

Components of the regimens studied have included an immunosuppresive agent with or without subsequent infusion of HLA-haploidentical marrow cells and with supplemental androgenic steroid theraphy.

Immunosuppresive agents have included cyclophosphamide, 6-methyl-prenisolone, ALG and ATG.

Although in vitro data suggest that hematologic

Table 3. Treatment with ATG and/or bone-marrow infusion and its outcome

Case No.	ATG dose (mg/kg × No. days) treatment	No. of B.M. cells ($\times10^8$/kg)	Donor	No. of transfusion (after ATG/BMT)		Latest blood counts			Duration of survival after treatment (days)	Response to treat ment
				RBC	platelet	Hb (g/dl)	WBC ($\times10^3$/mm^3)	platelets ($\times10^3$/mm^3)		
1	16 × 7	–	–	many	5		no change		330	–
2	16×10	4.5	mother	1	–	8.5	3.8	54	315	+
3	16×10	–	–	3	–	7.9	2.7	30	255	±
4	16×10	–	–	–	–	13.2	7.8	125	245	++
5	16×10	3.1	mother	2	2	10.4	5.6	76	180	+
6	16×10	–	–	5	3		no change		95	–

recovery may be the result of eliminating a population of T lymphocytes (possible suppressor T-cell) that have suppressed normal stem cell differentiation, conclusive in vovo data for this mechanism are lacking (Ascensao et al., 1976; Torok-storb et al., 1980; Bacigalupo et al., 1981; Warren et al., 1981). The study of bone marrow infusion following ALG therapy was first reported by Mathe et al. (1970). The intent of these initial study was to attain permanent engraftment of HLA-mismatched donor cells in aplasia patients.

No patient developed graft-versus-host disease (GVHD). Three of seven patients with aplastic anemia partially recovered marrow function.

An experimental rabbit model has been utilized by speck et al. (1971 and 1973) to examine the role of high dose immunosuppressive therapy with mismatched marrow. Aplasia was induced either by repeated injections of benzene or by a single injection of ^{32}P. when maximal peripheral pancytopenia and marrow hypocellularity occurred, antilymphocyte serum (ALS) was administered daily for four days.

Bone marrow was then infused from sex mismatched, randomly bred donors. A majority of animals in both groups achieved engraftment. Most animals exhibited split hematopoietic chimerism and none developed graft-verous-host disease.

Animals with benzene aplasia achieved prolonged engraftment (>100 days) whereas ^{32}p. rabbits rejected donor marrow in 5-13 weeks with subsequent return of antologous marrow function.

In vitor studies of a single patient with aplastic anemia were reported by Ascensao et al. (1976) and by Kagan et al. (1976).

They found that treatment of the patients marrow with ATG plus complement resulted in an increase in the ability of the patient's marrow to form colonies in soft agar. The first combined European study utilizing ATG was reported by speck et al. (1978).

Forty-one patients with severe aplastic anemia were treated a median of 8 months after diagnosis. Twenty-seven patients received ATG along and 14 received ATG plus marrow from a family member sharing one histocompatibility haplotype.

Fifty-seven percent (8/14) of those patients receiving ATG plus marrow are surviving greater than 200 days post-transplants.

None of these 8 are transfusion dependent. Of those receiving ATG alone, 52% (14/27) had improvement in their hematologic status and are surviving 75 to greater than 200 days.

Gluckman et al. (1979) reported a similar 35% response rate among 40 French patients with severe aplastic anemia treated with ATG and androgenes.

Five of these patients relapsed after an initial response to horse ATG therapy and were retreated with a second course of rabbit ATG; 4 of there patients responded to retreatment. Follow up ranged from 2 months to 2 years.

On the basis of there data, it was postulated that antilymphocyte serum and bone marrow infusion, by producing a transient graft, provided hematologic "protection" until spontaneous recovery could occur.

The major focus of this trial will be to identify those patients with a possible immune etiology of their aplastic anemia in children. The patients in this study who recovered after ATG therapy have had a varied course. The addition of haploidentical bone marrow infusion to ATG and androgens were associated with hematologic recovery. Long term follow up of these patients and further studies of this treatment are underway.

Toxicity of the ATG therapy (16 mg/Kg/dose $\times$ 10 doses) was tolerable. High fevers and chills were noted in all patients. Delayed symptoms included a "serum sickness like" symptom complex including intermittent fever, skin rash and arthralgias were noted in most cases. Platelet transfusion requirements during ATG therapy were also noted in three cases.

In general, the patients for whom bone marrow transplantation is not possible, can be offered ATG early in the course of their disease. Patients who have Only heplo-matched donors available may also be advised to consider ATG therapy with marrow infusion.

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