A Comparison of Jurkat Cell-Reactive Anti-T Lymphocyte Globulin and Fetal Anti-Thymocyte Globulin Preparations in the Treatment of Aplastic Anemia

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Key Words

Aplastic anemia · Lymphoglobulin® · Thymoglobulin® · ATG-Fresenius®

Abstract

Objective: The aim of this study was to investigate the success rate and effects on survival of different anti-thymocyte globulin (ATG) preparations in patients diagnosed with aplastic anemia. **Subjects and Methods:** Of the total 24 patients included in the study, 12 were male and 12 female with a median age of 44 years (range 16–72). Nine patients received Lymphoglobulin®, 7 Thymoglobulin® and ATG-Fresenius® (ATG-F). There was no significant difference between the three treatment groups in terms of severity of aplastic anemia. **Results:** The estimated 6-month survival rates for ATG-F, Lymphoglobulin and Thymoglobulin groups were 42.9, 77.8 and 71.4%, respectively. The difference in overall survival rates between groups was not significant, most likely due to the low number of patients. The most striking result was that none of the patients in the ATG-F preparation group showed any response to treatment. The ATG-F group was found to have a significantly inferior response rate ($p = 0.07$).

Conclusion: Our data showed that none of the patients responded to ATG-F treatment. Hence, despite the small number of the patients, we recommend that ATG-F should not be used for treatment of severe aplastic anemia.

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Introduction

Severe aplastic anemia is a life-threatening disease characterized by peripheral blood pancytopenia and hypocellular bone marrow. The prevalence of aplastic anemia is estimated at 1–2 per 1 million people with a higher incidence in some regions of Asia. Aplastic anemia is a disease of young adults (15–30 years), but a second peak in incidence has been reported in the sixth decade of life [1]. The mechanism of bone marrow failure is immune-mediated such that the suppression of hematopoiesis in aplastic anemia is probably secondary to activated cytotoxic lymphocytes [2]. Immunosuppressive therapy is a major treatment modality for severe aplastic anemia and anti-thymocyte globulin (ATG)/anti-lymphocyte globulin (ALG) is usually used. Cyclosporine is another immuno-

suppressive drug used for aplastic anemia therapy. The addition of cyclosporine led to higher hematologic response rates (60–70%) and more complete responses than observed with ATG alone [3]. Bone marrow transplantation remains the treatment of choice in patients if an appropriate family donor is available. Children have a low rate of graft-versus-host disease, which is cured by stem cell transplantation. Adults also successfully receive transplants, although the risk and severity of graft-versus-host disease and other transplant-related complications increase with age. ATG/cyclosporine combination therapy is considered the standard therapy for patients with severe or very severe aplastic anemia and transfusion-dependent nonsevere aplastic anemia, without suitable donors for stem cell transplantation [4].

Three different immunosuppressive therapy regimens, ALG, ATG and rabbit ATG (ATG-Fresenius®, ATG-F) are used as treatment of aplastic anemia. Thoracic duct lymphocytes are the antigens for ALG; thymocytes are the immunogens used for the production of ATG. Both appear to have much higher efficacy for treatment of aplastic anemia [5–7]. ATG-F is isolated from the serum of rabbits immunized with human T lymphoblasts from the human Jurkat cell line instead of children's thoracic duct lymphocytes or thymocytes. The thoracic duct lymphocyte ATG, a product called Lymphoser, is no longer produced. Both Thymoglobulin® and Lymphoglobulin®, produced with thymocytes of newborns, are used for stimulation, whereas ATG-F is produced with the cultured Jurkat cell line. Furthermore, there are species differences. Lymphoglobulin is a horse product whereas Thymoglobulin and ATG-F are rabbit products. There are, therefore, two main variables to be considered: animal species and stimulating cells. The therapeutic mechanisms of ATG/ALG are immunosuppressive, immunostimulatory and direct effect on hematopoietic stem cells, but the mechanism of ATG-F is restricted to an immunosuppressive effect [8]. Hence, this study was designed to investigate the effects on survival and success rate of different ATG preparations in patients diagnosed with aplastic anemia.

Subjects and Methods

The records of patients diagnosed with aplastic anemia at Hacettepe University Faculty of Medicine, Department of Internal Medicine, Hematology Clinic between January 1993 and January 2005 and who had received ATG treatment were evaluated. All patients (n = 24) were evaluated using established criteria of aplastic anemia prior to initiation of therapy. These criteria included bone marrow cellularity of <30%, peripheral blood pan-

Table 1. Patient characteristics

	ATG-F (n = 8)	LNF (n = 9)	TMG (n = 7)	p value
Age, years				
Median	27.5	35	53	NS
Range	16–62	17–65	25–72	
Gender, F/M	5/3	5/4	2/5	NS
Aplastic anemia severity				
Severe	8	7	7	NS
Nonsevere	0	2	0	
Response				
Complete	0	4	2	0.11
Partial	0	2	1	
None	4	1	3	
Early mortality <3 months	4	2	1	NS
Hemoglobin level, g/dl				
Median	8.9	7	9.7	NS
Range	4.5–11.9	2.4–11.5	5.4–11.2	
White blood cell count per mm ³				
Median	2,500	2,100	3,100	NS
Range	400–3,800	1,000–3,600	800–3,500	
Platelet count per mm ³				
Median	19,000	23,000	20,000	NS
Range	5,000– 134,000	3,000– 100,000	6,000– 110,000	

LNF = Lymphoglobulin; TMG = Thymoglobulin; nonsevere aplastic anemia = polymorphonuclear cells >0.5 g/l; severe aplastic anemia = polymorphonuclear cells <0.2 g/l; F = female; M = male; NS = nonsignificant.

cytopenia and absolute reticulocyte cellularity of <30% defined as hypocellularity, decreases in each of the three series, increases in the size and number of individual fat and stromal cells in the absence of atypical hematopoietic cell morphology, malignant infiltration, myelofibrosis and megaloblastoid features. In patients with severe aplastic anemia peripheral blood pancytopenia was present if two of three peripheral blood criteria were met: absolute neutrophil count <500 cells/mm³, platelet count <20,000 cells/mm³, reticulocyte count <40,000 cells/mm³ in the absence of other hematological disease. Moderate aplastic anemia was defined as pancytopenia not fulfilling the criteria for severe disease, absolute reticulocyte count being defined as <1% (corrected for hematocrit). There was no difference between the patients of the three groups in terms of severity of aplastic anemia.

Exclusion criteria were a follow-up duration of less than 3 months and having received another treatment within 3 months of ATG treatment. According to these criteria, 9 patients were excluded from the study. Nine patients received Lymphoglobulin, 7 Thymoglobulin, and 8 patients ATG-F. Patient characteristics are given in table 1. A total of 24 patients with a median age of 44 years (16–72), 50% of whom were male and 50% female, received the treatment.

Table 2. Response rates

	ATG-F (n = 8)	LNF (n = 9)	TMG (n = 7)	p value
Complete response	0	4	2	0.11
Partial response	0	2	1	
No response	4	1	3	
Follow-up time, days				
Total	329	500	480	
Median	3–1,668	10–2,488	66–2,992	
Range	5–1,668	10–2,480	61–2,825	
6-month survival rate, %	42.9	77.8	71.4	0.117

LNF = Lymphoglobulin; TMG = Thymoglobulin.

After premedication with diphenhydramine and acetaminophen, Thymoglobulin was administered at 2.5–5 mg/kg/day intravenously (i.v.), Lymphoglobulin at 15 mg/kg/day i.v., and ATG-F at 3–5 mg/kg/day (recommended doses after solid organ transplantation). All three preparations were administered for 5 consecutive days. In the ATG-F group, 1 patient received 7.5 and another 10 mg/kg/day for 7 consecutive days. Moderate doses (1 mg/kg/day) of prednisone or methylprednisolone were administered during the first 2 weeks to ameliorate the symptoms of serum sickness, and gradually reduced the following week. Cyclosporine was started on day 1 at 5 mg/kg/day by mouth in divided doses every 12 h. Dosing was adjusted to maintain a drug level of 200–400 ng/ml and was administered for at least 180 days. Treatment efficacy was classified as complete response (CR), partial response (PR) and no response (NR), respectively.

Statistical Analyses

Statistical analyses were performed using the SPSS statistical package (version 15.0, SPSS, Chicago, Ill., USA). The differences between survival curves were tested for statistical significance using the χ^2 test. Overall survival was determined from the time of entry into the study to total follow-up time. Patients who were lost to follow-up were eliminated at the time of the last visit. Response-to-treatment rates for all ATG preparations were evaluated using χ^2 test, and survival rates were evaluated using Kaplan-Meier and log-rank tests. A p value <0.05 was considered to be significant. The difference in overall survival rates between the groups was statistically insignificant (p = 0.117), but the significance lies in the fact that this is a very rare disease.

Results

Patient characteristics are given in table 1. The comparison of response rates among the three groups is given in table 2. Follow-up characteristics are given in table 3. For the ATG-F group, 4 patients had NR and 4 patients died during first 3 months of treatment. For the Lympho-

Table 3. Follow-up characteristics of all patients

Group	Follow-up time after first treatment, days	Result	Additional treatment-1	Additional treatment-2	Final outcome
ATG-F	5	death			death
ATG-F	7	death			death
ATG-F	22	death			death
ATG-F	65	death			death
ATG-F	126	NR			NR, alive
ATG-F	535	NR	ATG-F		death
ATG-F	1,085	NR	ATG-F		NR, alive
ATG-F	1,668	NR	ATG-F	AlloSCT	CR, alive
TMG	61	death			death
TMG	83	PR			PR, alive
TMG	99	NR			death
TMG	427	NR	LNF		NR, alive
TMG	731	CR			CR, alive
TMG	1,183	NR	AlloSCT		CR, alive
TMG	2,825	CR			CR, alive
LNF	10	death			death
LNF	40	death			death
LNF	92	NR	TMG		NR, alive
LNF	416	CR			CR, alive
LNF	497	CR			CR, alive
LNF	783	PR	LNF		PR, alive
LNF	1,373	PR	TMG		CR, alive
LNF	1,577	CR			CR, alive
LNF	2,480	CR			CR, alive

LNF = Lymphoglobulin; TMG = Thymoglobulin; AlloSCT = allogeneic stem cell transplantation; CR = complete response; PR = partial response; NR = no response.

globulin group 4 patients achieved CR, 2 achieved PR, 1 had NR and 2 died during the first 3 months. For the Thymoglobulin group, 2 patients achieved CR, 1 achieved PR, 3 had NR and 1 died during first 3 months. The estimated 6-month survival rates for the ATG-F, Lymphoglobulin and Thymoglobulin groups were 42.9, 77.8 and 71.4%, respectively. The most striking result was that none of the patients in the ATG-F preparation group showed any response to treatment. The ATG-F group had an inferior response rate (p = 0.07).

Of the 8 patients who received ATG-F treatment, 3 were administered a repeat ATG-F treatment. Of those 3 patients 1 died, the second had NR, and the third had CR following allogeneic stem cell transplantation. Of the 7 patients who received Thymoglobulin treatment: 1 received Lymphoglobulin in addition and had NR; another had CR following allogeneic stem cell transplantation. Of

the 9 patients who received Lymphoglobulin treatment, 2 received Thymoglobulin in addition, 1 of whom had NR while the other had CR. One patient who received repeat Lymphoglobulin treatment had PR (table 3).

Discussion

Among the patients treated with the three preparations Lymphoglobulin, Thymoglobulin and ATG-F, none of the patients treated with ATG-F showed any response. A previous study using Lymphoglobulin alone; Lymphoglobulin and cyclosporine; Lymphoglobulin, cyclosporine, recombinant human granulocyte-macrophage colony-stimulating factor and rhu erythropoietin (rhu-EPO); or rabbit ATG (ATG-F), cyclosporine, rhuGM-CSF and rhu-EPO showed that the response rate to ATG-F, cyclosporine, rhuGM-CSF and rhu-EPO (53%) was significantly lower ($p = 0.039$) than for the other treatment groups. Our finding was similar to that of Zheng et al. [9], who used ATG-F in combination with cyclosporine,

rhuGM-CSF and rhu-EPO. It should be pointed out that ATG-F is not an anti-T lymphocyte or thymocyte globulin. It is isolated from the serum of rabbits immunized with the human Jurkat cell line of T lymphoblasts instead of children's thoracic duct lymphocytes or thymocytes. Although limited, there are data indicating that these preparations have different mechanisms of action [8]. The therapeutic mechanisms of the classical agents are immunosuppressive, immunostimulatory and direct effects on hematopoietic stem cells. However, the mechanism of ATG-F is restricted to an immunosuppressive effect [8].

The limitations of our study are its retrospective design and small sample size. The low patient numbers were due to the fact that immunosuppressive treatment has only been indicated for cases not eligible for allogeneic transplant. Despite the limitations of this study, we believe that the use of ATG-F as a novel treatment for aplastic anemia should be challenged, since its efficacy is still under close scrutiny.

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