Treatment of acute graft-versus-host disease with low-dose, alternate-day antithymocyte globulin

Francesco Graziani, Maria Teresa Van Lint,
Alida Dominietto, Anna Maria Raiola,
Carmela Di Grazia, Teresa Lamparelli,
Francesca Gualandi, Stefania Bregante,
Merilù Fiorone, Barbara Bruno, Andrea Bacigalupo
Dipartimento di Ematologia, Ospedale San Martino, Genoa,
Italy

Background and Objectives. The efficacy of antithymocyte globulin (ATG) in the treatment of graft-versus-host disease (GvHD) is controversial. In the present study we report on the use of low dose ATG (thymoglobuline, Sangstat) and steroids in 28 patients with moderate to severe acute GvHD.

Design and Methods. Fifteen patients received ATG as first-line treatment within 14 days of the diagnosis of GvHD (median 8 days, range 4-13). Twelve patients received ATG as second-line therapy, more than 14 days after diagnosis (median 32 days, range 14 to 98). The proportion of patients with severe (grade III-IV) GvHD at the time of ATG therapy was 4/15 in the former group and 7/13 in the latter (p=0.1).

Results. On day 30 after ATG the overall proportion of responders was 80% in the group administered ATG early and 38% in those given it later (p=0.03). The overall actuarial 3-year transplant-related mortality was 40% vs 74% for the early vs late ATG groups (p=0.03); the actuarial 3-year survival was, respectively, 49% vs 23% (p=0.04). For patients with GvHD grade III-IV the actuarial 1-year TRM was 47% for those given ATG early, 87% for the late ATG group and 82% for a concurrent control group of 26 patients not treated with ATG.

Interpretation and Conclusions. In conclusion, ATG may be considered for early treatment of acute GvHD, within a few days from the onset of the disease. A prospective trial has been started to test whether, in this setting, low dose ATG with steroids is superior to steroids alone.
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Key words: allogeneic bone marrow transplantation, acute graft-versus-host disease, antithymocyte globulin.

Correspondence: Andrea Bacigalupo, MD, Divisione Ematologia 2 (PAD 5/II), Ospedale San Martino, largo Rosanna Benzi 10, 16132 Genoa, Italy. Phone: international +39.010.355469.
Fax: international + 39.010.355583.
E-mail: apbacigalupo@smartino.ge.it

Acute graft-versus-host disease (GvHD) is a major complication in patients undergoing allogeneic hematopoietic stem cell transplants (HSCT) especially with the increasing use of unrelated donors (UD).[1-3] In our Center the incidence of severe GvHD (grade III-IV) among patients receiving an HSCT from an UD, mismatched family members or a HLA-identical sibling is 21%, 10% and 7%, respectively. Once GvHD has developed it is unclear whether aggressive immunosuppression is beneficial. In a previous randomized trial we compared early therapy with 2 mg/kg/day of intravenous 6-methylprednisolone (6MPr) with 10 mg/kg/day 6MPr: there was no difference in response rate, transplant mortality or survival in the two groups.[4] In that study we showed that high dose 6MPr did not prevent the progression of acute GvHD to grade III-IV in 15% of patients treated early. We took these data to suggest that either 10 mg/kg/day is insufficient to control the immune reaction, or that alternative immunosuppressive agents should be tested in this disease.

ATG has been used in several trials to treat patients with acute GvHD.[5-8] Given as a single agent ATG does not seem to be more effective than 6MPr alone, thus combined treatment with steroids as first-line therapy has been explored.[8] A randomized trial comparing prednisone with antithymocyte globulin/prednisone as initial therapy for moderately severe acute GvHD showed that the combination of horse ATG/prednisone failed to improve acute GvHD and actually negatively affected survival by causing more infections.[9] In that study ATG was used at the conventional high dose (30 mg/kg/day) for five consecutive days.

Rabbit ATG (thymoglobuline) is used at lower doses than horse ATG (1/2 to 1/5). In addition recent experimental data suggest that rabbit ATG may induce apoptosis and T-cell unresponsiveness also at very low dose.[10,11] We therefore designed a

pilot study for treatment of acute GvHD grade II or greater, with low-dose, alternate-day rabbit ATG and methylprednisolone. We now report the outcome of this trial in 28 patients.

Design and Methods

Patients

Twenty-eight patients entered this trial. They all received an allogeneic BMT between December 1996 and June 2000; they all developed acute grade II-IV GvHD and were treated with low dose rabbit ATG. They had all failed to benefit from a first course of methylprednisolone. Patients were divided in two groups according to the timing of ATG therapy; one group started ATG within 14 days of the diagnosis of GvHD (median 8, range 4 to 13), while the second group started ATG more than 14 days after the diagnosis of GvHD (median 32, range from 14 to 98). The latter group comprised 7 relapsing patients, 3 non-responders and 3 with progressive GvHD. The median age was 26 years (range 20 to 53) and 41 years (range 20 to 61) in the early and late ATG group, respectively (Table 1). Underlying diseases were chronic myeloid leukemia (n=20), acute lymphoblastic leukemia (n=2), acute myeloid leukemia (n=2), myelodysplasia syndrome (n=2), myelofibrosis (n=1) and paroxysmal nocturnal hemoglobinuria (n=1) (Table 1). The conditioning regimen was total body irradiation (TBI)-based in 10 patients in the early ATG group and in 9 of the late ATG group (p=0.8), and was of conventional intensity. GvHD prophylaxis consisted of cyclosporine (CS) and methotrexate (MTX). ATG was used in the conditioning regimen as part of a randomized trial in three patients in each group at a total dose of 7.5 mg/kg.

GvHD treatment schedule

At the time of GvHD diagnosis all patients started 6MPr 2 mg/kg/die or 5 mg/kg/die. Patients received antithymocyte globulin at the dose of 1.25 mg/kg/every other day for 3 doses (n=7 and n=4) and for 5 doses (n=8 and n=9) in the early and late group, respectively. Treatment with 6MPr was continued together with the ATG.

Response criteria

We evaluated response to therapy by individual organs according to the current Seattle staging system. We compared acute GvHD grades on the day ATG therapy was started and then 10 and 30 days later, and identified patients whose GvHD resolved, improved, did not change or progressed. Response was assessed using an overall GvHD organ stage score described by Weisdorf *et al.*,[12]

Table 1. Patients' characteristics.

	Early ATG	Late ATG	p
Number of patients	15	13	
Recipient age median (range)	26 (20-53)	41 (20-61)	0.04
Donor age median (range)	40 (20-58)	39 (22-55)	0.3
Diagnosis			
CML	11	9	0.7
AL	2	2	–
MDS	1	1	–
Other	1	1	–
Disease phase	10/5	7/6	0.4
Early/advanced	10/5	7/6	0.3
Donor type: HLA identical sibling/other HLA identical sibling	6/9	5/8	0.9
MUD	6	5	0.6
Interval GvHD-ATG	8 (4-13)*	32 (14-98)	0.001
Follow-up	269 (56-1,463)*	158 (42-1,211)	

*Abbreviation: GvHD: graft-versus-host disease; ATG: antithymocyte globulin; *median interval in days (range).*

representing the sum of each acute GvHD organ stage (0 to 4) plus 1 point for upper gastrointestinal involvement, thus with a maximum possible score of 13 (0 to 4 in three organs plus upper gastrointestinal involvement). The GvHD score for each organ was added up on day zero of ATG therapy, then after 10 and 30 days. Response evaluation was based on clinical criteria: patients were divided into responders (improvement by at least one point without worsening in any other organs) and non-responders, these latter subdivided into those with stable disease (same sum score without worsening) or progressive disease (increased score or worsening in any organ).

Controls

Twenty-six patients were chosen for a subset analysis of grade III-IV GvHD. Their median age was 38 years (17-66), not different from that of patients in the early ATG group (p=0.2) or the late ATG group (p=0.4). The median year of transplant (p=0.5), diagnosis (p=0.9), phase of disease (p=0.1) and donor type (p=0.9) were also comparable.

Statistical analysis

χ^2 and Fisher exact tests were used together with the Mann Whitney rank sum test. Survival analyses were run according to Kaplan and Meier, and the log-rank test was used to evaluate differences between curves. The number cruncher software (NCSS, version 5.0; JL Hintze, Kaysville, UT, USA) was used to run the analyses.

Table 2. Clinical presentation of GvHD.

	Early ATG	Late ATG	p
Number of patients	15	13	
Interval BMT-GvHD: days	14 (7-39)*	8 (4-20)	0.001
Patients with GvHD II	11	6	–
Patients with GvHD III-IV	4	7	0.1
Patients with GvHD organ stage score 2-4	11	7	–
Patients with GvHD organ stage score 5-9	4	5	0.2

*Median and (range).

Table 3. Acute GvHD in individual organs in the early (A) and in the late (B) ATG group.

A	Skin	Liver	Gut
Grade 0	0	6	5
Grade I	4	5	7
Grade II	10	4	2
Grade III	1		1
Grade IV			
Total involvement	15 (100%)	9 (60%)	10 (66%)

B	Skin	Liver	Gut
Grade 0	0	3	4
Grade I	3	7	4
Grade II	9	2	2
Grade III			3
Grade IV	1	1	
Total involvement	13 (100%)	10 (77%)	9 (69%)

Results

Clinical characteristics of acute GvHD

The onset of acute GvHD occurred at median interval of 14 days after transplant (range 7 to 39) in the early ATG group and 8 days (range 4 to 20) in the late ATG group (p=0.001); ATG treatment was started at a median interval of 24 days after transplant (range 14 to 38) and 43 days (range 25 to 105) in the early and late ATG groups, respectively (Table 2). At the time of ATG therapy overall acute GvHD was scored as grade II (n=11 and n=6) and as grade III-IV (n=4 and n=7, p=0.1) in the early and late ATG groups respectively (Table 2). On day 0 of treatment eleven and seven patients had a total clinical score of 2-4 and four and five

patients had a score of 5-7 in the early and late ATG groups, respectively while the only patient with a score of 9 was in the late ATG group (p=0.2) (Table 2). All patients (100%) in both groups with acute GvHD had skin involvement, while nine (60%) and ten (77%) in the early and late ATG groups, respectively, had liver involvement (p=0.2), and ten (66%) and nine (69%) had gut involvement (p=0.6). Table 3A and Table 3B outline the severity of involvement of each organ on day zero of ATG therapy in the early and late ATG groups, respectively.

Response to therapy by individual organs

Responses (complete resolution + improvement) were seen in 47% and 40% of cases of skin GvHD in the early and late ATG group, respectively (p=0.5), in 55% and 57% of liver GvHD (p=0.6) and in 50% and 58% of gut GvHD (p=0.5). There was no change of GvHD in 46% and 40% cases of skin disease (p=0.5), 33% and 28% of liver involvement (p=0.6) and 30% and 42% (p=0.8) of gut disease in the early and late ATG groups, respectively. Progression was seen in 7% and 20% of skin GvHD (p=0.3), in 12% and 15% of liver GvHD (p=0.8), and in 20% and 0% of gut GvHD (p=0.3) in the early and late ATG groups, respectively.

Overall response

After 10 days the proportion of patients with improvement was 74% (n=11) in the early ATG group and 38% (n=5) in the late ATG group (p=0.06) (Table 4); the proportion of cases of stable GvHD was 13% (n=2) and 7% (n=1), and the percentage of worsened GvHD 13% (n=2) and 39% (n=5). Within 10 days of therapy 0% and 15% (n=2) in the 2 groups had died (Table 4) (p=0.1). The proportion of patients with worsened GvHD or who died was 13% (n=2) vs 54% (n=7) (p=0.02). The number of patients with GvHD stage score ≥ 4 was 7 in each group on day 0, and 1 vs 7 (p=0.008) on day +10 of treatment. After 30 days the proportion of responders was 80% (n=12) and 38% (n=5) in the early and late ATG groups, respectively (p=0.03), the proportion with stable GvHD was 0% and 15% (n=2), the proportion of patients whose disease worsened was 20% (n=3) and 23% (n=3) and the proportion of patients who died was 0% and 23% (n=3) (p=0.05). In the late ATG group three patients died within thirty days (Table 4). The proportion of patients with worsened GvHD or who died was 20% vs 46% (p=0.1).

Chronic GvHD

Among patients surviving 100 days limited chronic GvHD was diagnosed in 33% vs 0% and ex-

Table 4. Clinical response of GvHD to ATG therapy stratified according to the timing of treatment (early ATG, late ATG).

	Evaluation +10 days after ATG therapy			Evaluation +30 days after ATG therapy		
	Early ATG	Late ATG	p	Early ATG	Late ATG	p
Response of GvHD						
Improved	11 (74%)	5 (38%)	0.06[b]	12 (80%)	5 (38%)	0.03[b]
Stable	2	1		0	2	
Worsened	2	5		3	3	
Dead	0	2 (15%)	0.1[b]	0	3 (23%)	0.05[b]
Mean GvHD stage score[d]	2.2	4.1	0.002[a]	2.3	3.7	0.07[a]
N. of patients with GvHD stage score ≥ 4	1/7[c]	7/7	0.008[b]	3/7	4/7	0.4[b]

[a]Student's T test; [b]Fisher's exact test; [c]1 patient of the 7 with stage score ≥ 4 on day 0 of ATG therapy; [d]on day 0 of ATG, the mean GvHD score was 3.6 vs 4.4 (p=0.2).

tensive chronic GvHD in 66% vs 100% in the early and late ATG patients (p=0.05).

TRM and survival

The overall actuarial 3-year transplant-related mortality was 60%; it was 40% vs 74% for the early vs late ATG groups (p=0.03) (Figure 1). Four patients died of GvHD, two of leukemia and one patient of interstitial pneumonia in the early ATG group, while ten patients died of GvHD in the late ATG group. TRM correlated with response to treatment: it was 30% for responders (n=17) vs. 100% for non-responders (n=11) (p<0.0001). TRM was not influenced by donor type (HLA identical donor vs MUD: 42% vs 69%, p=0.3) or by recipient age (55% vs 57% for patients aged ≤37 or >37 years: p=0.6), but was related to disease phase (45% for early disease vs 85% for advanced disease, p=0.009). The actuarial 3-year survival was 49% vs 23% (p=0.04) in the early and late ATG groups, respectively (Figure 2).

Grade III-IV GvHD

To test whether results were different in patients with life-threatening GvHD (grade III-IV) we compared patients receiving early ATG (n=7, 29% of which grade IV), late ATG (n=11, 18% of which grade IV) and concurrent controls not receiving ATG (n=26, 19% grade IV). The crude TRM was 42%, 82%, 70% respectively (p=0.1), further suggesting little effect of ATG if given late beyond day +14.

Infections

Fifteen patients (65%) developed *de novo* CMV infection within two months after ATG treatment; there was no difference between the early and late ATG groups (66% vs 62%) (p=0.7); in the late group five had previously developed CMV reactivation and these cases were not considered as post-ATG
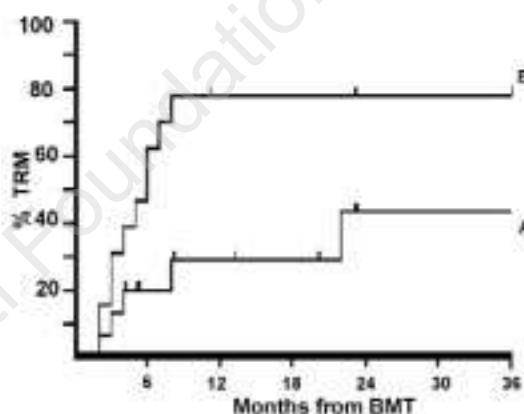

Figure 1. Actuarial 3-year TRM in 15 patients receiving ATG early (A= 40%) and 13 patients receiving ATG late (B= 74%) (p=0.03).
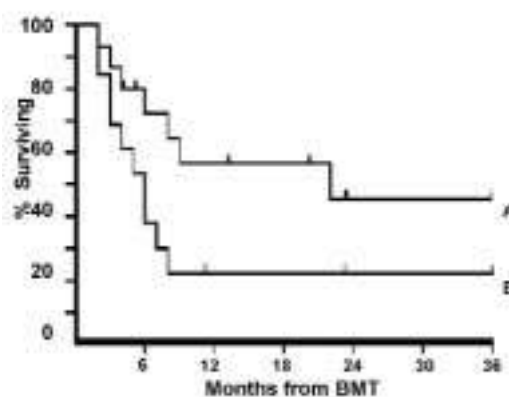

Figure 2. Actuarial 3-year survival in 15 patients receiving ATG early (A= 49%) and 13 patients receiving ATG late (B= 23%) (p=0.04).

complications.

Causes of death

Of the 28 patients 17 have died, 7 in the early ATG group and 10 in the late ATG group. GvHD was the leading cause of death (27% and 69% in the two groups, respectively) followed by infections (7% and 8%) and leukemia (13% and 0%, respectively).

Discussion

Treatment of moderate to severe acute GvHD is still unsatisfactory and the disease carries a high mortality. Corticosteroids are considered the cornerstone of GvHD treatment, and were reported to be more successful than ATG in 740 patients enrolled in a retrospective study.[13] A randomized study comparing corticosteroids with ATG in 37 patients showed no difference in response rate.[6] This study suggested that corticosteroids induced a more rapid response than ATG, and it was noted that 12 out of 17 patients given ATG required further therapy for GvHD, compared with 10 of 20 patients treated with corticosteroids.[6] It should be noted that in this study steroid treatment was more prolonged than ATG therapy.

A randomized study comparing low versus high dose 6MPr in 95 patients undergoing an HLA-identical BMT showed that early treatment of GvHD with high dose 6MPr did not improve the response rate as compared with low dose treatment and did not prevent evolution to GvHD grade III-IV.[4]

Secondary treatment of GvHD with ATG, following failure of first-line corticosteroids, appears to have limited efficacy in selected patients.[14] In a prospective study 42 MUD-BMT recipients were given steroids as first-line therapy, followed by ATG after prednisone failure in 22 of them.[15] Prednisone treatment led to improvement in 10 out of 41 (24%) of patients, while secondary treatment with ATG caused improvement in 4 out of 21 (19%). It was suggested that an extended previous corticosteroid treatment may select a clone of resistant T-cells that could be insensitive to a later corticosteroid treatment when grade II-IV acute GvHD developed.[15]

Because of these unsatisfactory data Cragg *et al.* performed a randomized trial comparing the combination of horse ATG/prednisone versus prednisone as initial treatment of GvHD.[9] They failed to show improved control of GvHD and obtained no significant difference in survival. In the ATG/prednisone arm there was an increased incidence of infections. The authors used an aggressive immunosuppressive therapy in the ATG/prednisone arm with high dose

ATG (15 mg/kg ATG *bid*) plus 20 mg/m^2 prednisone *bid* on each of 5 consecutive days.

In our study ATG was given as secondary treatment. The chosen dose was low, (1.25 mg/kg/every other day for 3 or 5 doses) for two reasons: the experimental data showing that low dose ATG induces T-cell apoptosis[10,11] and an attempt to test a small dose of ATG, therefore minimizing clinical immunosuppression and potentially fatal infections. There were two cohorts of patients: the first was treated early, within two weeks at a median GvHD-ATG interval of 8 days (range 4 to13) whereas the second group was treated later, beyond two weeks, at a median GvHD-ATG interval of 32 days (range 14 to 98). We found a significant difference in terms of overall response at 10 days (74% vs 38%, *p*=0.06) and at 30 days of treatment (80% vs 38%, *p*=0.03) in favor of the early ATG group. The average GvHD organ stage score in the 2 groups of patients recorded on day 0, day +10 and day +30 confirmed this result. The proportion of patients who had either died or had progressed on day +10 was significantly different (13% vs 54%, *p*=0.02). More importantly the overall TRM was significantly lower in the early ATG group than in the late ATG group (40% vs 74%, *p*=0.03) and survival improved (49% vs 23%, *p*=0.04). In order to test whether these results were biased by a different distribution of severe GvHD, we analyzed only patients with grade III-IV disease and compared them with concurrent controls, who developed grade III-IV GvHD and were not treated with ATG because of the individual choice of the attending physician. Transplant mortality in the early ATG, late ATG and no ATG group was 42%, 82%, and 70%, respectively. This suggests that ATG may be helpful if given early, within 14 days, especially in the presence of life-threatening grade III-IV GvHD, but seems to produce little effect when given beyond day 14, when compared to outcomes in patients not receiving ATG. In keeping with these data a recent study confirms that response and survival are superior in patients given ATG within 14 days.[16] In conclusion, we take these data to indicate that ATG should be considered early after the onset of acute GvHD, possibly at low doses, rather than later in the course of the disease. The number of patients is small and these data need to be confirmed in a prospective study. A prospective randomized trial is underway to test the efficacy of low dose ATG in combination with prednisolone compared to prednisolone only as first-line therapy of acute GvHD.

Contributions and Acknowledgments

FG collected the data, reviewed the literature, did the first analysis, and wrote the first draft of the manuscript. AD, AMR, TL, FG, CDG, SB were responsible for the clinical trial and the care of the patients. BB and MLF were responsible for data control and data quality. AB reviewed the manuscript , the data analysis, the discussion and the conclusions.

Disclosures

Conflict of interest: none.

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Peer Review Outcomes

Manuscript processing

What is already known on this topic

Horse ATG at 15-30 mg/kg 5 to 7 days does not add substantial benefit to front-line steroid treatment for acute GVHD. The improved control of GVHD under combined therapy is counterbalanced by a high frequency of severe infections

What this study adds

Rabbit ATG at low dose (1.25 mg/kg 3 to 5 days), instituted early as secondary treatment following steroid failure, allowed an adequate control of acute GVHD and improved survival in a substantial proportion of patients.

Potential implications for clinical practice

Early institution of Rabbit ATG at low dose plus steroids may be an effective therapy for acute GVHD after allogeneic stem cell transplantation. This hypothesis is currently being tested in a prospective randomized trial, comparing this combination to steroids alone as treatment for acute GVHD.

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