Rabbit-immunoglobulin G levels in patients receiving thymoglobulin as part of conditioning before unrelated donor stem cell transplantation

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Background and Objectives. The role of serum concentrations of rabbit antithymoglobulin (ATG) in the development of acute graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT) with unrelated donors is unknown.

Design and Methods. We determined the serum concentration of rabbit immunoglobulin-G (IgG) using an enzyme linked immunosorbent assay in 61 patients after unrelated donor HSCT. The doses of ATG ranged between 4 and 10 mg/kg. The conditioning consisted mainly of cyclophosphamide and total body irradiation or busulfan. Most patients received GVHD prophylaxis with cyclosporine and methotrexate.

Results. The rabbit IgG levels varied widely in each dose group. The levels of rabbit IgG gradually declined and could still be detected up to five weeks after HSCT. We found a correlation between the grade of acute GVHD and the concentration of rabbit IgG in serum before the transplantation (p=0.017). Patients with serum levels of rabbit IgG >70 µg/mL before HSCT ran a very low risk of developing acute GVHD grades II-IV, as compared to those with levels <70 µg/mL (11% vs. 48%, p=0.006).

Interpretations and Conclusions. The measurement of rabbit IgG levels in patients receiving ATG as prophylaxis against GVHD after HSCT may be of value in lowering the risk of severe GVHD.

Key words: ATG, GVHD, BMT, thymoglobulin, rabbit-IgG.

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The outcomes of unrelated donor hematopoietic stem cell transplantation (HSCT) have substantially improved during the past decade. The reason for this is more sophisticated tissue typing and perhaps also better supportive care. However, graft-versus-host disease (GVHD) is a major cause of morbidity and mortality using unrelated donors.[1,2] The risk of graft failure is also higher in unrelated donor HSCT. By adding antithymocyte globulin (ATG) to the pre-transplant conditioning protocol, acute GVHD and early mortality of unrelated donor HSCT have been reduced to levels similar to those of matched related HSCT.[3,4] Many different doses and types of ATG are used in transplant centers. The optimal dose of the various ATG preparations, as regards the prevention of graft failure and GVHD, is not yet fully understood. However, the dose of ATG may not be the sole cause of the level of *in vivo* depletion of donor T-cells. The distribution in the patient's tissues and elimination also affect the level and rate of T-cell depletion. Thymoglobulin® is produced by immunizing pathogen-free rabbits with fresh human thymocytes. The γ immunoglobulin (Ig) fraction contains polyclonal antibodies against multiple cell surface antigens.[5-7] Thymoglobulin binds to T cells and, to a lesser extent, to B cells, monocytes, macrophages and neutrophils.[5-7] It also binds to platelets and red blood cells, albeit at low levels. It produces its effect via antibody-dependent cell-mediated cytotoxicity (ADCC), induction of apoptosis and down-regulation of cell surface antigens.[8] The elimination half-life after 1 day of treatment has been shown to be 2-3 days.[9] Since 1990, we have mainly used four doses of thymoglobulin as part of the pre-transplant conditioning therapy in unrelated donor HSCT[10] and have found a dose-dependent effect of ATG on acute GVHD grades II-IV and III-IV.[10] Here we have analyzed rabbit IgG levels in 61 patients and compared these with the dose of ATG given and GVHD.

Design and Methods

Patients

This is a study of 61 patients who received thymoglobulin (Genzyme, Cambridge, MA, USA) as part of their conditioning before allogeneic HSCT from a matched unrelated donor. The transplants were performed between April 1998 and June 2003 at the Center for Allogeneic Stem Cell Transplantation, Karolinska University Hospital, Stockholm, Sweden. Most patients had a hematologic malignancy. Of the 61 patients, 35 were male and 26 female The median age of the series was 35 years (range 1-61) and 14 (23%) of the patients were less than 18 years old. Table 1 shows the patients' and donors' characteristics.

Conditioning

The conditioning regimen in 6 patients consisted of cyclophosphamide (60 mg/kg/day for 2 days), followed by 10 Gy single fraction total body irradiation (TBI), with the lungs shielded to receive no more than 9 Gy.[11] Another 21 patients received fractionated TBI (4 Gy/day for 3 days) combined with cyclophosphamide. As an alternative, busulfan, 4 mg/kg/day for 4 days, and cyclophosphamide was given to 25 patients.[11] Nine patients were given reduced conditioning with fludarabine (30 mg/m^2) for 3-5 days combined with 2 Gy TBI (n=2), busulfan total dose 8 mg/kg (n=3) or cyclophosphamide total dose 60 mg/kg (n=4).[12-14]

GVHD prophylaxis

Post-transplant immunosuppression consisted of cyclosporine and a short course of methotrexate in 53 patients.[15] Other protocols included cyclosporine combined with prednisolone (n=3) or mycophenolate mofetil (n=3). The combination of rapamycin and prograft was used in two patients. During the first month, blood cyclosporine levels were kept at 200-300 ng/mL. After three or six months depending on whether the patients had malignant or non-malignant disease, respectively, the cyclosporine dose was lowered by 25% every alternate month and discontinued after six (malignant) or 12-24 months (not malignant diseases) in the absence of GVHD.

Thymoglobulin doses

The total dose of thymoglobulin ranged between 4 and 10 mg/kg and was given at a dose of 2 mg/kg/day for 2-5 days (Table 1).[10] The last dose of thymoglobulin was always given the day before transplantation. Pre-treatment with 500-1000 mg of methylprednisolone and 2 mg of i.v. anti-histamine was given to most patients as prophylaxis against adverse events before the first dose of thymoglobulin. In those patients who did develop adverse events, 100-200 mg of hydrocortisone was also given.

Table 1. Patients' and donors' characteristics for the 61 patients who received rabbit ATG (thymoglobulin) as part of their conditioning before unrelated donor HSCT.

	Thymoglobulin
N	61
Diagnosis	
Acute myeloid leukemia	18
Myelodysplastic syndrome	5
Acute lymphoid leukemia	10
Chronic myeloid leukemia	11
Other malignancy	5
Non-malignant disease	8
Solid tumor	2
Disease stage (early/late)	34/25
Recipients' gender (M/F)	35/26
Recipients' age	35 (1-61)
Donors' gender (M/F)	39/22
Donors' age	38 (21-54)
NC dose (×10^8/kg)	6.5 (1-54.5)
SC source (BM/PBSC)	28/33
Conditioning	
Cy+TBI 10 Gy	6
FTBI+Cy	21
Bu+Cy	25
RIC	9
GVHD prophylaxis	
MTX+CsA	53
CsA+Prednisolone	3
CsA+MMF	3
Rapamycin+Prograft	2
G-CSF post-HSCT	31 (51%)
Thymoglobulin dose	
4 mg/kg	14
6 mg/kg	21
8 mg/kg	15
10 mg/kg	11

Early disease: complete remission, chronic phase 1, Late disease: > complete remission, chronic phase 1, NC dose: nucleated cell dose, SC: stem cells; BM: bone marrow, PBSC: peripheral blood stem cells, Cy: cyclophosphamide; TBI: total-body irradiation; FTBI: fractionated TBI; Bu: busulfan; RIC: reduced intensity conditioning; MTX: methotrexate; CsA: cyclosporine; MMF: mycophenolate mofetil; G-CSF: granulocyte colony-stimulating factor.

Rabbit IgG detection

Samples of serum were collected before the transplant (n=58)(after the last dose of thymoglobulin), at one week (n=53), two weeks (n=19), three weeks (n=31), four weeks (n=9) and 5 weeks (n=12) after HSCT. These samples were kept at -20°C pending analysis. They were then thawed and analyzed using

an enzyme-linked immunosorbent assay (ELISA). Each serum sample was tested in duplicate in three 5-fold dilution steps. Samples and controls were diluted in phosphate-buffered saline containing 0.05% Tween 20 (Polysorbatum 20, Apoteksbolaget, Sweden) and 0.1% human AB serum. Polystyrene microtiter plates (Nunc polysorp, Denmark) were coated overnight with 2 μg/mL swine anti-rabbit IgG antibodies (Z0400, DAKO Cytomation, Denmark) at +4°C in 0.1 M carbonate-bicarbonate buffer (pH 9.6). We used rabbit serum as a positive control, human AB serum as a negative control. Thymoglubulin was used as a standard at 1000, 100, 50, 25, 12.5 and 6 ng/mL; 100 μL of standards, controls and samples were added and incubated for 3 hours at room temperature. Between the various incubation steps, the wells were rinsed four times with saline containing 0.05% Tween 20. Finally, 100 μL of alkaline conjugated goat anti-rabbit IgG (D0487, DAKO Cytomation, Denmark) were added in a dilution of 1:3000 for 1 hour at room temperature. After a final wash, disodium *p*-nitrophenyl phosphatase in 1 M diethanolamine buffer was added and absorbance was measured at 405 nm, using a microplate reader (Vmax, Molecular devices, USA). The serum concentrations were calculated using SOFT MAX PRO 3.0 software. The rabbit serum IgG concentration was 14.4±2.5 mg/mL (mean ± SD) in 14 experiments.

HLA typing

All patient and donor pairs were typed, using PCR-SSP high resolution typing for HLA class I and class II antigens16. Most patient and donor pairs were HLA-A, -B and –DRβ1-compatible. Sixteen patients had an allele level mismatched donor.

Stem-cell source and supportive care

Thirty-three patients received stem cells from peripheral blood and 28 from bone marrow.[17] Before apheresis, all donors of peripheral blood stem cells were treated with granulocyte colony-stimulating factor (G-CSF) (Rhône-Poulenc Rorer, Lyon, France or Amgen-Roche Inc., Thousand Oaks, CA, USA) 10 μg/kg/day for 4 to 6 days subcutaneously once a day. The growth factor was given to 31 (51%) of the patients from day 10 after HSCT until the absolute neutrophil count exceeded >0.5×10^9/L for two consecutive days.[18]

Diagnosis and treatment of GVHD

Acute and chronic GVHD were diagnosed on the basis of clinical symptoms and/or biopsies (skin, liver, gastrointestinal tract, or oral mucosa) according to standard criteria.[19,20] The patients were treated for grade I acute GVHD with prednisolone, starting at 2 mg/kg/day, and then the dose was tapered off after the initial response. In more severe cases, ATG, methylprednisolone, methotrexate or psoralen and ultraviolet A light (PUVA) was used. Chronic GVHD was initially treated with cyclosporine and steroids. If no response occurred, some patients were given total lymph node irradiation, PUVA or extracorporeal PUVA.

Definitions

Bone marrow aspirates were performed and the morphology analyzed 3, 6, and 12 months and then yearly after HSCT. Leukemia relapse was defined as more than 5% blasts in bone marrow aspirates or the presence of extramedullar leukemia cells i.e., extramedullar relapse. Bacteremia was defined by the first positive blood culture related to a febrile episode during the first 30 days after transplantation.

Cytomegalovirus (CMV) reactivation was determined with polymerase chain reaction (PCR) in leukocytes weekly.[21] Engraftment was defined as a stable absolute neutrophil count >0.5×10^9/L for three consecutive days and platelet engraftment as platelet counts >30×10^9/L for seven consecutive days without transfusions.

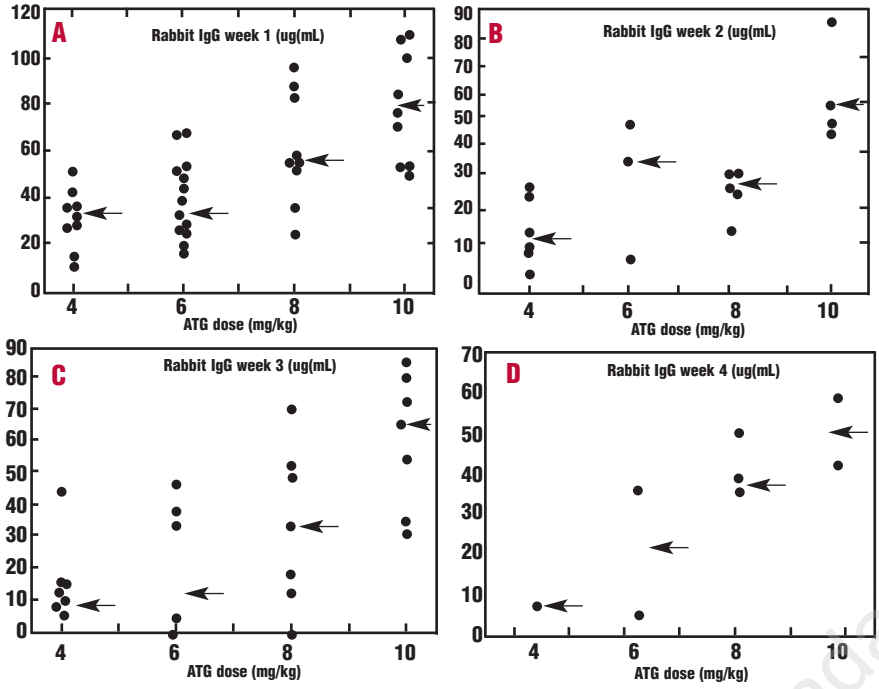
Statistics

The analysis was performed in August 2004. Separate statistical analysis were performed for each endpoint (GVHD, transplant-related mortality and relapse). The incidence of each event was estimated non-parametrically. Patients were censored at the time of relapse or last follow-up.[22] Relapse and non-relapse mortality were competing events. Their incidence rates were therefore estimated using a non-parametric estimator of cumulative incidence curves. Predictive analyses were based on the proportional hazard model for subdistribution of competing risk. Analyses were performed using the cmprsk package (developed by Gray, June 2001), Splus 6.2 software and Statistica software. The Kruskal-Wallis test and Mann-Whitney U test, whichever was appropriate, were used to compare the levels of rabbit IgG between groups. Factors with a p value <0.1 in the univariate analysis were included in the multivariate analysis, i.e. ATG dose, ATG concentration, the patient's age and sex, stage of the disease, nucleated cell dose, the donor's age and sex, stem-cell source, growth factor treatment after HSCT, HLA-match and type of conditioning therapy.

Results

Thymoglobulin dose

Correlations were found between the dose of thymoglobulin given and the rabbit IgG levels in serum



Figures 1. Plot of rabbit IgG levels in patients A) 1 week, B) 2 weeks, C) 3 weeks and D) 4 weeks after HSCT in the four thymoglobulin dose-groups. Arrows indicate median values.

after HSCT (r=0.67, r=0.72 and r=0.67 at week 1, week 2 and week 3 after HSCT, respectively. In the samples taken after the last thymoglobulin dose, but before the transplantation, this correlation was lower (r=0.27). However, the levels of rabbit IgG varied among the patients in each dose group (Figure 1). The levels of rabbit IgG slowly declined from day 0 to 5 weeks after HSCT, with the same difference between the four dose-groups (Figure 2). The greatest fall was seen between day 0 and 1 week after transplantation. At week 1 after transplantation, we found a median of 65% of the day 0 level of rabbit IgG in serum, as compared to 34%, 20%, 15% and 13% of the day 0 levels at 2, 3, 4 and 5 weeks. The estimated half-life of rabbit IgG was about 10 days. We detected rabbit IgG up to 5 weeks after administration of ATG in 10 of 12 patients (median 8.0 μg/mL, range 0-45.8 μg/mL).

No role of leukocyte counts and graft content. No correlations were noted between the leukocyte counts immediately before the first dose of ATG and the levels of rabbit IgG on day 0 in each dose group. The median leukocyte counts immediately before ATG treatment were 4.6×10^9/L and 2.9×10^9/L in patients with acute GVHD grades 0-I and II-IV, respectively (p=0.3). No correlations were found between the nucleated cell count in the graft and the decline in the rabbit IgG level between day 0 and week 1. The median nucleated cell-count in the graft in patients with acute GVHD grades 0-I and II-IV were 5.8×10^8/kg (range 0.6-54.5) and 10.0×10^8/kg (1.0-22.8), respectively (p=0.15).

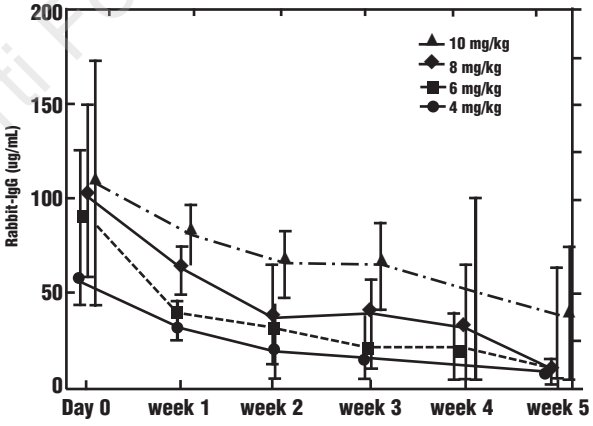


Figure 2. Rabbit IgG level kinetics in the four thymoglobulin dose-groups. Mean ± 95% CI.

Engraftment

No correlations were observed between the thymoglobulin dose or the rabbit IgG levels and the day of neutrophil and platelet engraftment. Two patients rejected their grafts. In both of them the levels of rabbit IgG were higher at transplant (100 and 214 μg/mL) and one week after transplantation (83 and 98 μg/mL), p=0.09 and p=0.05 as compared to a median of 65 μg/mL (18-293 μg/mL) and 45 μg/mL (12-109 μg/mL), respectively, in those who did not reject their grafts.

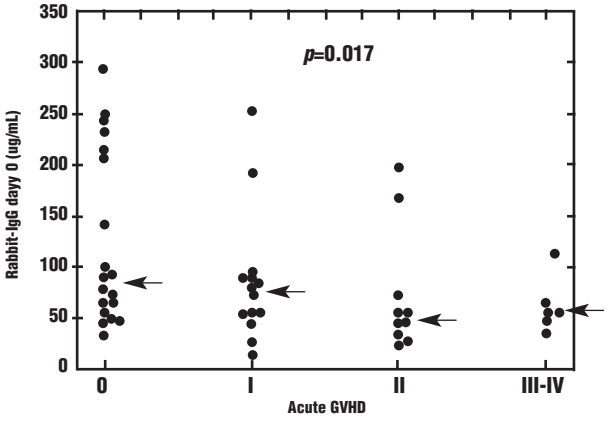


Figure 3. Plot of rabbit IgG levels on day 0 in patients with various grades of acute GVHD. Arrows indicate median values. Acute GVHD developed at a median of 24 (range 10-72) days after HSCT.

Graft-versus-host disease

In the whole series (n=61), the cumulative incidence of acute GVHD grades II-IV and III-IV was 33% and 12%, respectively. A dose-dependent effect on severe acute GVHD was seen. The cumulative incidence of acute GVHD grades III-IV was 21%, 10%, 7% and 8% in patients receiving 4, 6, 8, and 10 mg/kg of ATG. We also found a correlation between the grade of acute GVHD and the level of rabbit IgG in serum before (p=0.017) and one week after transplantation (p=0.16) (Figure 3). Patients with rabbit IgG levels >70 μg/mL before HSCT (day 0) ran a lower risk of developing moderate-to-severe acute GVHD (3/27, 11%), as compared to those who had levels <70 μg/mL (16/31, 48%, p=0.006)(Figure 4A). The incidence of acute GVHD grades III-IV was 4% and 19% in patients with greater and less than 70 μg/mL of rabbit IgG on day 0 (p=0.08). A significant cut-off level in samples from 1 week after HSCT was also found, i.e. 5/28 patients with rabbit IgG levels >45 μg/mL developed acute GVHD grades II-IV compared with 13/25 of patients with levels <45 μg/mL (p=0.01) (Figure 4B). None of the 17 patients with levels >70 μg/mL on day 0 and levels >45 μg/mL at one week developed acute GVHD grades II-IV. In contrast, three of the seven patients with levels >70 μg/mL on day 0 and <45 μg/mL at 1 week developed grades II-IV GVHD (p<0.001)(Table 2). Results from the univariate analysis of risk factors for acute GVDH grades II-IV are shown in Table 3. In the multivariate analysis, we found that rabbit IgG levels <70 μg/mL on day 0 (OR 6.42, CI 1.90-17.8, p=0.003) and peripheral blood stem cells (OR 3.19, CI 1.22-8.33, p=0.017) were independent risk factors for the development of acute GVHD grades II-IV. There were no differences between patients with greater or less than 70 μg/mL rabbit IgG at day 0 with respect to other known risk factors for GVHD.

The cumulative incidence of chronic GVHD was 26%. Only one out of 12 patients with rabbit-IgG levels >100 μg/mL at day 0 developed chronic GVHD compared to 9/40 patients with rabbit-IgG levels <100 μg/mL. However, this difference was not statistically significant (p=0.27).

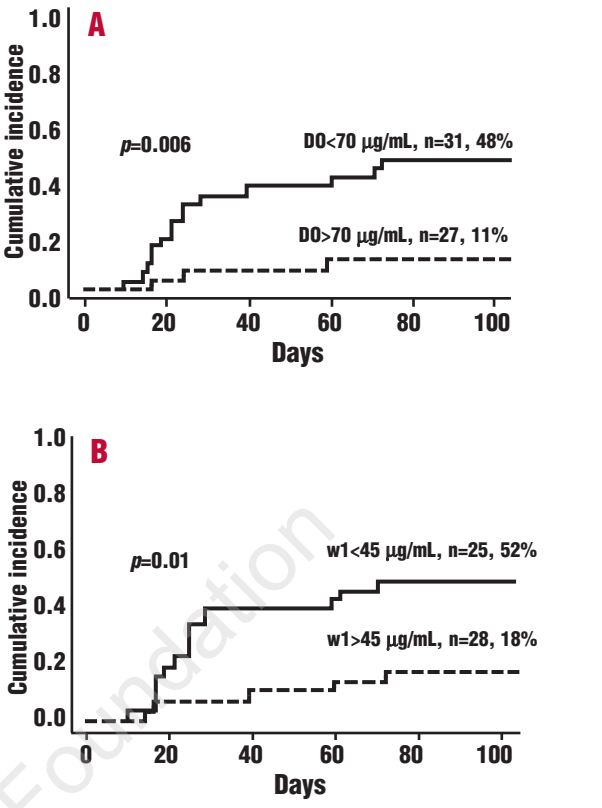


Figure 4. A. Cumulative incidence of acute GVHD grades II-IV in HSCT patients with rabbit IgG levels above or below 70 μg/mL on day 0. B. Cumulative incidence of acute GVHD grades II-IV in HSCT patients with rabbit IgG levels above or below 45 μg/mL one week after HSCT.

Table 2. Rabbit IgG levels in four groups divided according to thymoglobulin dose. Absolute numbers of patients in each group are given. Numbers in brackets indicate patients who developed acute GVHD grades II-IV.

Rabbit IgG levels/ATG dose	*4 mg/kg*	*6 mg/kg*	*8 mg/kg*	*10 mg/kg*
<70 μg/mL on day 0	10 (7)	11 (5)	7 (2)	3 (2)
>70 μg/mL on day 0	4 (1)	10 (2)	8 (0)	5 (0)
Patients with > 70 μ/mL	4 (1)	3 (2)	0	0
on day 0 and:	0	5 (0)	7 (0)	5 (0)
<45 μg/mL at 1 week				
>45 μg/mL at 1 week				

Infections

Bacteremia was diagnosed in 29 patients (48%) and CMV reactivation in 32 (52%). No significant correlations between infections and rabbit IgG levels in serum were found in this series. Seven of 10 (70%) patients with rabbit-IgG levels >150 μg/mL at day 0 had a CMV infection compared to 25/48 (52%) with rabbit-IgG levels <150 μg/mL (p=ns).

Transplant-related mortality

A trend towards a better transplant-related mortality at one year was seen in patients with rabbit IgG levels >45 μg/mL one week after HSCT than in those with <45 μg/mL (22% vs.32%, p=0.11).

Discussion

ATG was infused before the transplantation to achieve *in vivo* T-cell depletion in the patient and the graft and thereby reduce the risk of graft failure and GVHD. The effectiveness of ATG as prophylaxis for GVHD in recipients of matched grafts from unrelated donors has been described elsewhere.[23-29] In these studies, various doses of ATG have been used with very different incidences of GVHD. In recent studies, we showed that there was a dose-dependent effect of ATG on the incidence of acute GVHD.[10,30] A correlation between ATG dose and acute GVHD has also been shown by Mothy *et al.*[30] In these studies low-dose ATG (<6 mg/kg and <7.5 mg/kg, respectively) was associated with more acute GVHD. However, little is known about the correlation between the doses of ATG and the serum concentration of ATG and the effect on GVHD. In this study, we determined the levels of total rabbit IgG in serum before and after HSCT in 61 patients given four different doses of thymoglobulin, and correlated these levels with the development of GVHD.

We found that although the correlation was good between the dose given and the level of rabbit IgG in serum, the levels varied considerably in each dose group. The patients with lower levels before, and at one week after transplantation, developed acute GVHD grades II-IV. This effect was seen regardless of the dose of thymoglobulin. In those given the higher doses (8-10 mg/kg), 10 of 23 patients had low levels of rabbit IgG (<70 μg/mL) before transplantation. Four of these 10 patients developed moderate-to-severe acute GVHD, while none of the 13 patients with high rabbit IgG levels (>70 μg/mL) developed acute GVHD (Table 2). In those given low doses of thymoglobulin (4-6 mg/kg), 12/21 patients with low levels (<70 μg/mL) of rabbit IgG before HSCT developed acute GVHD, while 3/14 patients with high levels (>70 μg/mL) developed acute GVHD. These findings suggest that the levels of rabbit IgG before infusion of the graft are more important for the development of acute GVHD grades II-IV than is the dose of ATG given. In the multivariate analysis, we found that the rabbit IgG levels on day 0 (<70 μg/mL) and a peripheral blood stem cell graft were independent risk factors for acute GVHD grades II-IV. The lev-

Table 3. Results from univariate analysis of factors associated with acute GVHD grades II-IV in 61 patients receiving thymoglobulin as part of their conditioning prior to unrelated donor HSCT.

Factor	*OR*	*95% CI*	*p value*
ATG dose			
6-10 mg/kg	1		
4 mg/kg	2.62	0.75-9.16	0.12
Rabbit-IgG day 0			
>70 μg/mL	1		
<70 μg/mL	7.5	1.80-31.1	0.005
Rabbit-IgG week 1			
>45 μg/mL	1		
<45 μg/mL	4.4	1.24-15.6	0.02
Sex			
Male	1		
Female	4.0	1.26-12.7	0.02
Age			
Continuous	0.98	0.95-1.02	0.26
Disease stage			
Early	1		
Late	2.17	0.69-6.85	0.18
Match grade			
Match	1		
Subtype MM	1.33	0.39-4.49	0.64
Nucleated cell dose			
Continuous	1.12	0.95-1.09	0.64
CD34 dose			
Continuous	1.01	0.91-1.02	0.54
Donor sex			
Male	1		
Female	1.75	0.57-5.55	0.31
Donor age			
Continuous	1.01	0.93-1.07	0.94
Conditioning			
TBI-based	1		
Busulfan	0.39	0.12-1.34	0.13
Conditioning			
Myeloablative	1		
RIC	0.54	0.10-2.98	0.47
Stem-cell source			
Bone marrow	1		
Peripheral blood	2.70	0.85-8.62	0.09
G-CSF post-HSCT			
No	1		
Yes	0.95	0.32-2.84	0.93

els of rabbit IgG were more important than the dose of infused thymoglobulin. For this reason it could be appropriate to monitor the rabbit IgG levels in patients given ATG as prophylaxis against GVHD. Patients with low levels may benefit from an additional dose of ATG after HSCT.

Rabbit ATG can be detected up to 1 month after administration[31] and can negatively affect T-cell and immune reconstitution,[25,29,32] increasing the risk of infectious complications. An association between high doses of ATG and a higher risk of fatal infections has been reported.[32,33] In our study, we detected rabbit IgG up to 5 weeks after infusion. We could not confirm the finding of more infections and deaths due to infections in patients with higher levels of rabbit IgG. However, our study included only 61 patients, which may have been too few to detect a significant effect. We found a trend towards more non-relapse deaths in those with low rabbit IgG levels one week after HSCT (19% vs. 36%, p=0.11). This is probably due to the correlation between acute GVHD and transplant-related deaths.

Another drawback of ATG treatment may be a higher incidence of relapse.[23,25,26] Some researchers have shown that patients with chronic myeloid leukemia who receive a T-cell-depleted graft run a high risk of relapse.[35] We have also found a correlation between ATG treatment and an increase in the risk of relapse in patients with chronic myeloid leukemia.[23] Higher doses of ATG may be associated with an effective *in vivo* T-cell depletion of the graft, which increases the risk of relapse. No conclusions can be drawn from the present study because the number of patients with chronic myeloid leukemia was too small. However, this increased risk of relapse was not supported by data from the studies by Kröger *et al.*[34] and Duggan *et al.*[24] in which ATG was very effective in reducing the incidence of GVHD without affecting the incidence of relapse. It should, however, be noted that Kröger *et al.* used ATG-Fresenius. In the present study, we found no correlation between the levels of rabbit IgG and the incidence of relapse. In a previous study examining various doses of thymoglobulin, we found no correlation between the dose of thymoglobulin and relapse.[10]

It is noteworthy that the two patients who rejected their grafts had high levels of ATG at the time of transplant. It can be speculated that the high levels of ATG reduced the number of donor T-cells and paved the way for rejection in an immunized host.

Some data have shown that ATG can be detected up to one month after administration.[31] In our study, we found rabbit IgG 5 weeks after administration in 10/12 patients analyzed at this time, but the levels were low (median 8.0 μg/mL). This indicates that the immunosuppressive effect of ATG may persist for more than one month, at least when higher doses are given. However, we only determined the total rabbit IgG level and not active ATG, which is the fraction of ATG that retains the capacity to bind to human lymphocytes. A previous study showed that the latter has a shorter half-life than total ATG (7 days vs. 14 days).[36] In this study all patients received thymoglobulin divided into 2 mg/kg/day doses, with the last dose given the day before transplantation. A larger last dose, the day before HSCT, may increase rabbit Ig levels early after HSCT. However, this schedule may increase toxic side-effects by massive cytokine release[37] and a increased risk of rejection and infectious complications cannot be excluded.

In conclusion, measuring rabbit IgG levels in patients on ATG as prophylaxis against GVHD after HSCT may be of value in evaluating the risk of severe GVHD. Patients with low levels may benefit from an additional dose of ATG after HSCT.

MR: designed the study, analyzed and interpreted the results, drafted the article and approved the final version; BS: developed the method to analyze rabbit-Ig, analyzed all samples, revised the article for important intellectual content and approved the final version. The authors reported no potential conflicts of interest.

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