Chapter 2

Effect of Antithymocyteglobulin on quantitative immune recovery and graft-versus-host disease after partially T cell depleted bone marrow transplantation: a comparison between recipients of matched related and matched unrelated donor grafts

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Abstract

The effect of Antithymocyteglobulin (ATG) on quantitative immune recovery and graft-versus-host-disease (GVHD) after partially T cell depleted (TCD) bone marrow transplantation (BMT) was analysed in 59 and 32 recipients of grafts from matched related donors (MRD) and matched unrelated donors (MUD), respectively. The conditioning regimen was similar in all patients, except for ATG which was given only to MUD recipients. Thirteen MUD patients were treated with high-dose (20 mg/kg) ATG and 19 with low-dose (8 mg/kg) ATG. During the post-transplant period both CD3+, CD4+ and CD8+ T cell numbers and the incidence of acute and chronic GVHD were significantly lower in MUD recipients compared to MRD patients. MUD recipients treated with high-dose ATG showed the worst T cell and subsets recovery. These data suggest that ATG, often used as part of conditioning regimens in recipients of TCD grafts from MUDs, contributes to the very severe and prolonged T cell deficiency that is typical of these patients. On the other hand, it effectively reduces incidence and severity of GVHD.

Introduction

Allogeneic bone marrow transplantation (BMT) is an established treatment modality for adult patients with haematologic malignancies. However, 70% of patients who might benefit from allogeneic BMT lack an HLA-identical sibling donor (matched related donor, MRD). Therefore, there is an increasing use of HLA-matched unrelated donors (MUD). Unfortunately, transplant-related mortality (TRM) after MUD-BMT is much higher than after MRD-BMT, which is largely due to an increased incidence of opportunistic infections and graft-versus-host disease (GVHD)[1]. The increased incidence of opportunistic infections suggests that after MUD-BMT immune reconstitution is much more impaired, compared to BMT from a matched sibling donor. Several factors may account for the impaired immune reconstitution, such as HLA disparities, the increased incidence of GVHD and the use of more intensive immunosuppression to prevent or treat GVHD.

Here, we set out to unravel the contribution of some of these immunodeficiency inducing factors by studying the recovery of T cell subsets, NK cells and B cells after BMT in patients treated with partially T cell depleted (TCD) grafts from either a MRD or MUD. Both patient groups received a fixed low number of T cells in the graft and a similar conditioning regimen except for Antithymocyteglobulin (ATG) that was only given to recipients of MUD transplants.

Methods

Patients Between August 1997 and March 2001 allogeneic bone marrow transplantation was performed in 104 patients. Thirteen patients could not be analysed; 10 because of early death (< 2 months post-transplant), 1 received donor lymphocytes < 2 months post-transplant, 1 repopulated with blasts, 1 had a graft rejection. In total 91 patients, receiving either bone marrow from a MRD (n=59) or from a MUD (n=32), were studied. Patients with acute leukaemia in first complete remission, chronic myeloid leukaemia in first chronic phase, untreated severe aplastic anaemia were considered low-risk. All patients with other diseases were considered high-risk. The procedures were performed at the Department of Haematology of the University Medical Centre Utrecht. Patients were treated according to

clinical protocols approved by the local investigation review board after informed consent was obtained.

Transplantation procedure Transplantation procedure, monitoring and pre-emptive treatment of CMV reactivations, diagnosis and treatment of CMV disease, HLA typing and partial TCD of grafts were performed as described[2]. In brief, the conditioning regimen consisted of cyclophosphamide (60 mg/kg/day for 2 days), followed by total body irradiation (600 cGy/day for 2 days) with partial shielding of the lungs. ATG (Thymoglobulin™, Sangstat, Amstelveen, the Netherlands) was infused to MUD patients before cyclophosphamide was given. Thirteen patients received a dose of 4 mg/kg/day intravenously for 5 days. Due to a change in national treatment protocols ATG dose was lowered to 2 mg/kg/day for 4 days from April 1999. Nineteen patients received ATG low-dose.

Immunophenotyping Three colour FACS analysis (FACScan, Becton Dickinson Immunocytometry Systems (BDIS), San Jose, CA) was performed on heparin-anticoagulated venous blood. To 100 μL of undiluted blood a mixture of three antibodies was added consisting of either CD3-Cy5 (Beckman Coulter, Mijdrecht, The Netherlands), CD4-FITC (BDIS) and CD8-PE (BDIS); CD45-Cy5 (Beckman Coulter), CD3-FITC (BDIS) and CD19-PE (BDIS) or CD45-Cy5, CD3-FITC and a mixture of CD16-PE and CD56-PE (BDIS). After a 20 minute incubation at room temperature the erythrocytes were lysed using FACS lysing solution (BDIS), washed once with wash solution consisting of PBS supplemented with 1% bovine serum albumin and sodiumazide (0.01%), resuspended in 0.5 ml wash solution and analysed by flow cytometry. In every sample a minimum number of 15,000 events were acquired. List mode data were analysed using attractor software (BDIS). Percentages of T (CD3+) , B (CD19+) and NK cells (CD3-, CD16+ and CD56+) in the CD45+ leukocyte population were calculated. Based on the CD3/CD4/CD8 staining the distribution of CD4+ and CD8+ cells within the CD3+ T cell compartment was determined. Normal values were derived from McNerlan et al[3].

Statistics Data are expressed as mean ± SEM. Mean differences between patient characteristics were assessed by Students t-test or Chi-square analysis. Mean differences concerning T, B and NK cell recovery were tested using repeated-measures (RM) analysis and analysis of variance (ANOVA) with group (MRD vs MUD), risk status, CMV serostatus, acute GVHD (aGVHD) and chronic GVHD (cGVHD) as 'between-subjects' or 'fixed' factors, respectively, and age as covariate. All cell counts were log-transformed in order to meet the basic conditions on which the theory of RM-analysis and ANOVA is leaning. ANOVA was used

next to RM analysis, since RM analysis excluded too many cases (due to missing values). Both RM analysis and ANOVA gave comparable results. Levene's test was performed to find out whether the assumption of equality of variance could be maintained. Residuals were checked for normality by means of the Kolmogorov-Smirnov (KS) statistic. The KS-statistic in all cases showed values < 0.2 for the log-transformed cell count residuals. Calculations were performed using SPSS/PC + 10.0 (SPSS Inc, Chicago Il, USA).

Results

Patient characteristics Patient characteristics are described in Table 1. MRD recipients were significantly older (42 ± 1.3 vs 32 ± 1.8; $p<0.001$), received less T cells (1.4 ± 0.1 vs 2.7 ± 0.3 x 10^5/kg; $p<0.001$) and suffered more acute (grade II-IV) and chronic (extensive) GVHD (54% vs 19% and 30% vs 6%; $p<0.01$) compared to MUD recipients.

Immune recovery Analyses with group (MRD vs MUD), age, risk status, patient CMV serostatus, aGVHD and cGVHD as factors revealed that only 'group' and 'CMV serostatus' significantly influenced T cell and subsets recovery during the 6 months post-transplant period. In the MRD group markedly higher values were measured during this period, which was significant by repeated-measures analysis (CD3+ and CD4+ T cells: $p<0.01$; CD8+ T cells: $p<0.05$) and by analysis of variance (Figure 1). During the whole 6 months period no normal values of CD4+ cells were reached in both patient groups and of CD3+ cells in MUD recipients. Patient CMV serostatus also influenced T cell reconstitution. Patients with a positive CMV serostatus showed significantly higher T cell and subsets counts post-transplant compared to CMV-seronegative recipients ($p<0.01$). No interaction between 'group' (MRD and MUD) and 'patient CMV serostatus' was observed. B cell and NK cell recovery was not influenced by any of the factors tested. The incidence of fatal infectious complications was 19% in MUD recipients compared to 12% in MRD recipients (ns).

A subgroup analysis among MUD recipients revealed that none of the aforementioned factors significantly influenced immune reconstitution. MUD recipients treated with high-dose ATG showed a worse CD3+ and CD8+ T cell recovery compared to MUD recipients treated with low-dose ATG, although not significant (Figure 2).

Table 1 Patient characteristics

	MRD (n=59)	MUD (n=32)	P
Age, yr (range)	42 (18-56)	32 (18-52)	p<0.001
Diagnosis			
ALL	3	7	
AML	6	7	
CML	9	11	
SAA	0	4	
MM	16	0	
NHL	11	1	
MDS	0	2	
CLL	1	0	
CMV serostatus R/D			
+/+	20	6	ns
+/-	6	6	
-/+	11	4	
-/-	22	16	
Risk status (%)			
Low	29 (49)	16 (50)	ns
High	30 (51)	16 (50)	
T cell count in graft			
(x 10^5/kg)	1.4 ± 0.1	2.7 ± 0.3	p<0.001
GVHD (%)			
Acute			p=0.001
I/no	27 (46)	26 (81)	
II-IV	32 (54)	6 (19)	
III-IV	3 (5)	0	
Chronic			p=0.008
Lim/no/ne	41 (70)	30 (94)	
Ext	18 (30)	2 (6)	

SAA = severe aplastic anaemia; MM = multiple myeloma; R/D = recipient/donor; Lim = limited; Ext = extensive; ne = non evaluable.

Figure 1

B, T and NK cell numbers during the post-transplant period in recipients of MRD transplants compared to recipients of MUD transplants.
Shaded area represents normal values.
**=p<0.01

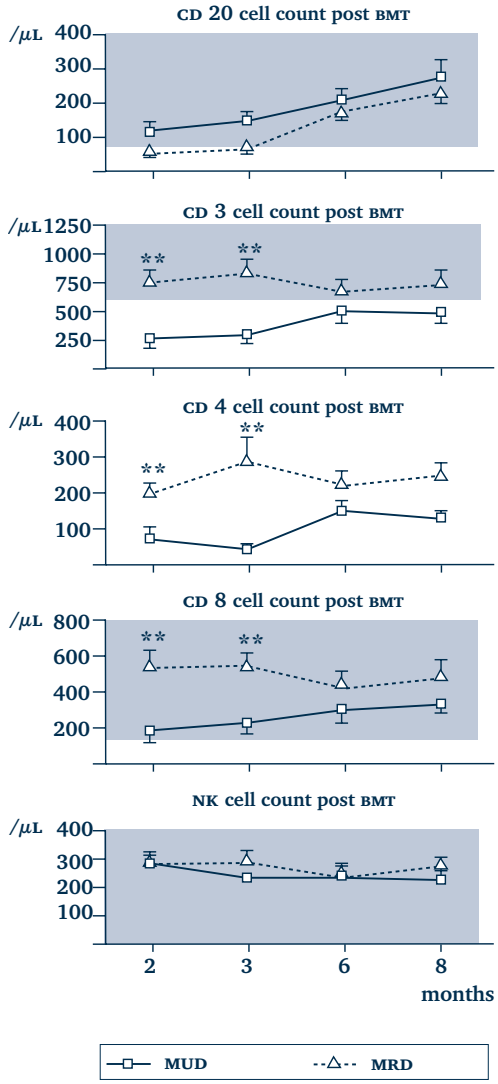
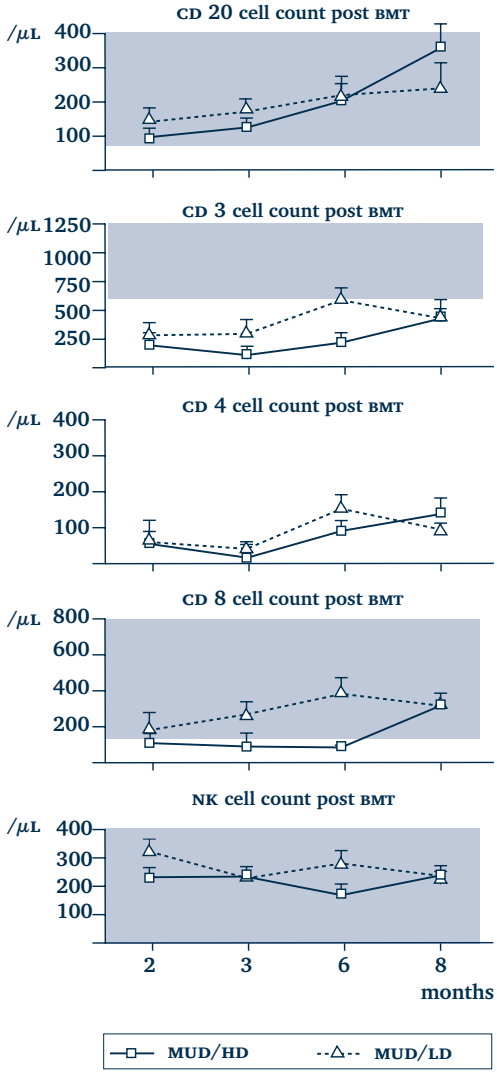


Figure 2

B, T and NK cell numbers during the post-transplant period in recipients of MUD transplants. Patients pretreated with low-dose ATG (MUD/LD) compared to patients pretreated with high-dose ATG (MUD/HD).
Shaded area represents normal values.



Discussion

The results of our study showed a markedly impaired quantitative T cell reconstitution during the first 6 post-transplant months in the group receiving BMT from a MUD compared to patients receiving transplants from an HLA-identical sibling. MUD recipients treated with high-dose ATG had the worst T cell and subsets recovery. These differences between MRD and MUD recipients might explain the well known increased incidence of infectious complications in MUD patients[1].

Two factors may account for the disturbed immune reconstitution among our MUD recipients compared to MRD patients: use of ATG and HLA disparities.

Since ATG could be detected in sera at least during 2 months post-transplant with 25% of the initial peak concentration at day 48 [4], it is hypothesized that ATG may severely impair T cell recovery, which was recently shown to be dose dependent[5].

It is also speculated that T and B cell recovery is related to the degree of HLA matching between host and donor. HLA disparities may alter the capacity of lymphoid progenitors of donor origin to mature within the host bone marrow or thymus. On the other hand, HLA disparities stimulate the occurrence of GVHD. Therefore, patients receiving grafts from unrelated or mismatched related donors will always be treated with more intensive immunosuppression compared to recipients of MRD grafts. A well known characteristic of GVHD itself is a defect in the development of donor derived T cells, which leads to a long lasting T cell deficiency state[6,7]. This entanglement of factors makes it impossible to solely analyse the impact of donor type on immune recovery. Despite this, efforts to analyse the influence of donor type were made in several studies[8-10]. In the first report a more prolonged and profound CD3+, CD4+ and CD8+ lymphopenia was seen in MUD recipients compared to MRD recipients. However, the proliferative T cell response to PHA was comparable between the two groups. Furthermore, interference of GVHD with the observed impaired T cell subset recovery in MUD recipients could not be excluded[8]. In the other studies, no effect of donor type was observed[9,10]. Recently, immune recovery in unrelated cord blood transplant (CBT) recipients was analysed and compared to data from recipients of MRD grafts. T cell receptor (TCR)-rearrangement excision circles (TREC) levels were comparable between the two groups, while TCR diversity was normalized earlier in CBT recipients[11]. Two other reports[12-13] did study the patterns of CD4+ and CD4+ CD45RA+ T cell recovery and restoration of TCR diversity in recipients of TCD grafts from MUDs and MRDs, respectively.

The results in both groups were highly comparable in these two reports. One might conclude from these results, that HLA disparities not necessarily result in an altered maturation of lymphoid progenitors of donor origin in the host thymus.
Based on these data we consider ATG to be the most important negative factor in the reconstitution of T cells and there subsets in our MUD recipients. On the other hand, ATG resulted in a lower incidence and severity of GVHD.

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References

1 Marks DI, Cullis JO, Ward KN, Lacey S, Syzdlo R, Hughes TP, Schwarer AP, Lutz E, Barrett AJ, Hows JM et al. Allogeneic bone marrow transplantation for chronic myeloid leukemia using sibling and volunteer unrelated donors. Ann Intern Med 1993; 119: 207-214.

2 Meijer E, Dekker AW, Rozenberg-Arska M, Weersink AJL, Verdonck LF. Influence of Cytomegalovirus-seropositivity on outcome after T cell depleted bone marrow transplantation: contrasting results between recipients of grafts from related and unrelated donors. Clin Infect Dis 2002; 35: 703-712.

3 McNerlan SE, Alexander HD, Rea IM. Age-related reference intervals for lymfocyte subsets in whole blood of healthy individuals. Scand J Clin Lab Invest 1999; 59: 89-92.

4 Finke J, Bertz H, Schmoor C, Veelken H, Behringer D, Wasch R, Kunzmann R, Heidecker L, Lang H, Meyer-Konig U, Mertelsmann R. Allogeneic bone marrow transplantation from unrelated donors using in vivo anti-T-cell globulin. Br J Haematol 2000; 111: 303-313.

5 Duval M, Pedron B, Rohrlich P, Legrand F, Faye A, Lescoeur B, Bensaid P, Larchee R, Sterkers G, Vilmer E. Immune reconstitution after haematopoietic transplantation with two different doses of pre-graft antithymocyte globulin. Bone Marrow Transplant 2002; 30: 421-426.

6 Atkinson K. Reconstruction of the haemopoietic and immune systems after marrow transplantation. Bone Marrow Transplant 1990; 5: 209-226.

7 Dulude G, Roy DC, Perreault C. The effect of graft versus host disease on T cell poduction and homeostasis. J Exp Med 1999: 189: 1329-1341.

8 Small TN, Papadopoulos EB, Boulad F et al. Comparison of immune reconstitution after unrelated and related T cell depleted bone marrow transplantation: effect of patient age and donor leukocyte infusion. Blood 1999; 93: 467-480.

9 Lewin SR, Heller G, Zhang L, Rodrigues E, Skulsky E, van den Brink MR, Small TN, Kernan NA, O'Reilly RJ, Ho DD, Young JW. Direct evidence for new T-cell generation by patients after either T-cell-depleted or unmodified allogeneic hematopoietic stem cell transplantations. Blood 2002; 100: 2235-2242.

10 Weinberg K, Blazar BR, Wagner JE, Agura E, Hill BJ, Smogorzewska M, Koup RA, Betts MR, Collins RH, Douek DC. Factors affecting thymic function after allogeneic hematopoietic stem cell transplantation. Blood 2001; 97: 1458-1466.

11 Talvensaari K, Clave E, Douay C, Rabian C, Garderet L, Busson M, Garnier F, Douek D, Gluckman E, Charron D, Toubert A. A broad T-cell repertoire diversity and an efficient thymic function indicate a favorable long-term immune reconstitution after cord blood stem cell transplantation. Blood 2002; 99: 1458-1464.

12 Dumont-Girard F, Roux E, van Lier RA, Hale G, Helg C, Chapuis B, Starobinski M, Roosnek E. Reconstitution of the T-cell compartment after bone marrow transplantation: restoration of the repertoire by thymic emigrants. Blood 1998; 92: 4464-4471.

13 Roux E, Dumont-Girard F, Starobinski M, Siegrist CA, Helg C, Chapuis B, Roosnek E. Recovery of immune reactivity after T-cell-depleted bone marrow transplantation depends on thymic activity. Blood 2000; 96: 2299-2303.