Biol Blood Marrow Transplant 20 (2014) 1156–1162

High Serum Level of Antithymocyte Globulin Immediately before Graft Infusion Is Associated with a Low Likelihood of Chronic, But Not Acute, Graft-versus-Host Disease

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Article history:
Received 29 November 2013
Accepted 7 April 2014

Key Words:
Antithymocyte globulin
Acute GVHD
Chronic GVHD

A B S T R A C T

Rabbit antithymocyte globulin (ATG) is administered during transplant conditioning to decrease the risk of both acute graft-versus-host disease (aGVHD) and chronic graft-versus-host disease (cGVHD). Here we evaluated the relationship between the serum concentration of ATG (capable of binding to lymphocytes) immediately before graft infusion (day 0) or on day +7 or +28 post-transplantation and the development of aGVHD or cGVHD. We studied 180 patients whose conditioning included 4.5 mg/kg antithymocyte globulin (ATG; Thymoglobulin). For aGVHD, we found no association with ATG levels on day 0. Nevertheless, high day +7 and +28 ATG levels were associated with a low likelihood of aGVHD. For cGVHD, high ATG levels at all 3 time points (days 0, +7, and +28) were associated with a low likelihood of cGVHD. In conclusion, high-dose ATG administration at the time of graft infusion appears to inhibit the development of cGVHD, but not aGVHD; however, higher ATG levels on days +7 and +28 are associated with lower rates of both aGVHD and cGVHD.

INTRODUCTION

The addition of rabbit antithymocyte globulin (ATG) to pretransplantation conditioning has been shown to decrease the incidence of acute graft-versus-host disease (aGVHD) and chronic graft-versus-host disease (cGVHD) in recipients of both unrelated and related donor grafts [1-11]. Two brands of rabbit ATG are available: Thymoglobulin (Genzyme/Sanofi, Cambridge, MA), produced from sera of rabbits immunized with human thymocytes, and ATG-F (Fresenius, Bad Homburg, Germany), produced from sera of rabbits immunized with Jurkat T cells. In both cases, ATG is produced by purification of IgG from the immune sera.

ATG is polyclonal and contains antibodies against antigens expressed on lymphocytes, other leukocytes, and nonleukocytes [12-15]. It is generally believed that the fraction of antibodies within ATG that can bind to lymphocytes is the functional (ie, anti-GVHD) fraction. Consistent with this idea, we have reported that high serum levels of this fraction on days +7 and +28 were associated with a low likelihood of both aGVHD and cGVHD [16]. We hypothesized that day 0 levels may be even more strongly associated with GVHD than day +7/+28 levels, because the day 0 levels should not be influenced by the variability in ATG elimination between day 0 and day +7/+28. The variability in elimination could be related to, for example, differences in graft content of cells that can adsorb ATG (on target antigens or Fc receptors) or differences in the number of recipient cells that can adsorb or eliminate ATG.

The primary objective of the present study was to evaluate the associations between the day 0, +7, and +28 ATG levels and aGVHD/cGVHD. The secondary objective was to evaluate associations between the day 0, +7, and +28 levels and relapse, cytomegalovirus (CMV) reactivation, post-transplantation lymphoproliferative disorder (PTLD), and death.

PATIENTS AND METHODS

Patients and Transplantation

We studied 185 consecutive recipients of allogeneic filgrastim-mobilized peripheral blood stem cells (PBSCs) for a hematologic malignancy who consented to participate in the study. All patients received ATG (Thymoglobulin) as part of pretransplantation conditioning. All transplantations were performed between December 2008 and December 2012. Patients who failed to engraft (n = 3), relapsed (n = 0), or died (n = 2) before day +30 were excluded; their ATG levels on days 0, +7 and +28 were not markedly different compared with the 180 patients included in the study, data for whom we report here. For 177 of these 180 patients, conditioning consisted of fludarabine 250 mg/m^2, busulfan ~12.8 mg/kg (pharmacokinetically adjusted), and ATG 0.5 mg/kg on day -2, 2.0 mg/kg on day -1, and 2.0 mg/kg on day 0 (total, 4.5 mg/kg) [7]. The last dose of ATG (day 0 dose) was given before graft infusion. Total body irradiation (TBI; 4 cGy) was also administered to 133 patients [17]. Table 1 shows patient and donor characteristics.

Post-transplantation GVHD prophylaxis consisted of cyclosporine from day -1 up to 3 months post-transplantation, at a starting dose of 2.5 mg/kg every 12 hours and later targeting trough serum levels of 200 to 400 g/L and methotrexate on days +1, +3, +6, and +11 (first dose, 15 mg/m^2 IV; next 3 doses, 10 mg/m^2 IV; the last dose was omitted in rare patients with airway-jeopardizing mucositis) [7]. No antifungal prophylaxis was given routinely until 2010; thereafter, fluconazole was given on days 0 to +28. Routine antibacterial prophylaxis was not provided, except for trimethoprim-sulfamethoxazole for pneumocystis prophylaxis given up to 6 months post-transplantation or longer in cases of cGVHD requiring systemic therapy. Viral prophylaxis consisted of acyclovir or valacyclovir for up to 2 years

Financial disclosure: See Acknowledgments on page 1161.

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1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2014.04.007

Table 1
Patient and Donor Characteristics

Characteristic	Value
Total patients, n	180
Patients with day 0 serum samples, n	152
Patients with day 7 serum samples, n	164
Patients with day 28 serum samples, n	160
Patient age at transplantation, yr, median (range)	50 (18-66)
Donor age at transplantation, yr, median (range)	36 (13-68)
Patient sex, M/F, n	104/76
Donor sex, M/F, n	116/64
Diagnosis, n	
Acute lymphoblastic leukemia	35
Acute myelogenous leukemia	68
Chronic lymphocytic leukemia	15
Chronic myelogenous leukemia	7
Myelodysplastic syndrome	21
Non-Hodgkin lymphoma	13
Myelofibrosis	10
Other hematologic malignancies	9
Disease risk stage, n*	
Good	94
Poor	86
Donor type, n	
HLA-matched sibling	67
Others	113
HLA match (-A, -B, -C, -DRB1, and -DQB1), n	
10/10 allele-matched	153
8-9/10 allele-matched	27
Conditioning regimen, n	
Flu + Bu + ATG + TBI	131
Flu + Bu + ATG	46
Flu + Cy + ATG + TBI	2
Cy + Bu + ATG	1
Acute GVHD† grade, n	
Grade 0	81
Grade I	51
Grade II	26
Grade III	15
Grade III-IV	1
Grade IV	5
Median day of onset of grade II-IV aGVHD (range)	56 (15-99)
Chronic GVHD,‡ n	
None	84
NNST	20
NST	48
Not applicable§	28
Median onset day of chronic GVHD NST (range)	126 (89-446)
Number of patients with CMV reactivation above threshold for preemptive therapy	45
Median day of onset of CMV reactivation (range)	43.5 (20-175)
Number of patients with PTLD	15
Median day of onset of PTLD (range)	55.5 (33-408)
Number of patients with relapse	33
Median day of onset of relapse (range)	182 (39-946)
Median day of follow-up for death (range)‖	448 (31-1448)
Median day of follow-up for death in patients who did not die (range)	566 (68-1448)
Median day of follow-up for relapse and nonrelapse death (range)¶	422 (31-1448)
Median day of follow-up for relapse and nonrelapse death in patients who did not relapse or die (range)	547 (59-1448)
Median day of follow-up for GVHD, CMV reactivation and PTLD (range)**	387 (31-1448)
Median day of follow-up for GVHD, CMV reactivation and PTLD in patients who did not develop graft failure, relapse, secondary malignancy, or death	564 (59-1448)

Flu indicates fludarabine; Bu, busulfan; TBI, total body irradiation; Cy, cyclophosphamide.

* Good risk disease/stage includes primary acute leukemia in first remission, chronic myelogenous leukemia in first chronic phase, and myelodysplasia with <5% marrow blasts. All other diseases/disease stages were considered poor risk.

† aGVHD occurred in 66 of 113 (58.4%) unrelated transplant recipients and in 34 of 65 (52.3%) matched sibling transplant recipients ($P = .4297$, chi square test).

‡ cGVHD occurred in 40 of 100 (40%) unrelated transplant recipients and in 28 of 56 (50%) matched sibling transplant recipients ($P = .2269$, chi square test).

§ Patients who developed second malignancy, relapse, or death or were lost to follow-up before day 100.

‖ Patients were followed until death or until the last day known to be alive.

¶ Patients were followed until they developed relapse or death or were lost to follow-up.

** Patients were followed until they developed graft failure, second malignancy (including PTLD), relapse, or death, or until they were lost to follow-up (defined as the last day on which medically meaningful information was available).

post-transplantation or longer in cases of cGVHD requiring systemic therapy and a preemptive CMV strategy [18].

Measurement of ATG Levels

Blood was drawn from patients on day 0 (within 15 minutes before graft infusion), day +7 (range, day +6 to day +8), and day 28 (range, day +24 to day +36) post-transplantation. Serum was stored in tightly sealed vials at minus 80°C until ATG level determination. We measured ATG levels (capable of binding to lymphocytes) using the flow cytometry–based assay developed by Kakhniashvili et al. [19] with minor modifications [16]. In brief, standards of known ATG concentrations, ranging from 20 to 0.0098 mg/L were prepared by serial 2-fold dilution. Peripheral blood mononuclear cells from a healthy volunteer were incubated with patient serum or an ATG standard. The cells (coated with ATG) were then labeled with phycoerythrin-conjugated goat anti-rabbit IgG. After flow cytometry data acquisition, lymphocytes were gated by forward and side scatter characteristics. Phycoerythrin fluorescence was measured for each standard and for each patient serum sample included in the run. Plotting ATG levels of standards versus the median channel of phycoerythrin fluorescence generated a standard curve, from which patient ATG levels were then extrapolated.

Definitions of Outcomes

aGVHD and cGVHD were categorized according to National Institutes of Health (NIH) criteria [20]. A minority of patients with insufficient information in their charts for unequivocal categorization of post–day +100 GVHD as cGVHD versus late aGVHD were classified as having cGVHD. aGVHD was graded in accordance with the 1994 consensus conference [21]. Chronic GVHD was graded as none, not needing systemic therapy (NNST), or needing systemic therapy (NST). "Any cGVHD" refers to cGVHD NNST or NST. Relapse, death, and nonrelapse death were defined using standard criteria. CMV reactivation was defined as CMV DNAemia above our institutional threshold for preemptive antiviral therapy (25,000 IU/mL plasma) or CMV disease. PTLD was defined as an illness with signs or imaging results consistent with PTLD (eg, fever not due to other causes, lymphadenopathy, splenomegaly, mass) with Epstein-Barr virus (EBV) DNAemia >400 copies/μg of leukocyte DNA or >40,000 copies/mL whole blood or histological evidence of PTLD, including in situ hybridization for EBV-encoded RNA.

Statistics

ATG levels in patients with versus patients without grade II-IV aGVHD, grade III-IV aGVHD, any cGVHD, cGVHD NST, relapse, death, nonrelapse death, CMV reactivation, and PTLD were compared using the Mann-Whitney-Wilcoxon (MWW) test. For outcomes for which ATG levels appeared to differ significantly between patients with and those without the outcome ($P \leq .05$, MWW test), we proceeded to multivariate analysis. Using log-binomial regression models, we determined whether patients with ATG levels above the cutoff had a higher/lower likelihood of the outcome compared with patients with ATG levels below the cutoff (multivariate analysis adjusting for confounding factors known to be associated with the outcome). The cutoff ATG levels were determined using the receiver operating characteristic (ROC) curve as the cutoff associated with the highest sum of sensitivity and specificity. To avoid having too small patient groups