

ORIGINAL ARTICLE

Glutathione S-transferase T1-null seems to be associated with graft failure in hematopoietic SCT

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Hematopoietic SCT (HSCT) from HLA-matched donors is sometimes complicated by GVHD or graft rejection, because of mismatched mHA. This study presents data suggesting the involvement of glutathione S-transferase theta-1 (GSTT1), a phase II detoxifying enzyme encoded by *GSTT1*, in Ab-mediated rejection of HSCT in children with congenital hemoglobinopathies (CHs). Mismatch of *GSTT1*, which often features a deletion polymorphism variant, can have major consequences in solid organ transplantation outcome. In liver transplantation, it has been shown to lead to *de novo* hepatitis, whereas in kidney transplantation, chronic allograft rejection has been documented. In this study on 18 children with CH who underwent HSCT, five cases of graft rejection occurred, all in GSTT1-null patients, four of which featured anti-GSTT1 antibodies. The data suggest that when GSTT1-null patients are transplanted with a GSTT1-positive graft, rejection due to an Ab-mediated immune response against GSTT1 displayed on transplanted stem cells may take place. Thus, it seems that detection of anti-GSTT1 antibodies in patients with a GSTT1-null genotype before transplantation may be predictive of graft rejection in the event of a GSTT1-positive donor.

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Introduction

Hematopoietic SCT (HSCT) is widely used to treat a broad range of malignant (for example, leukemia) and nonmalignant

diseases (for example, thalassemia). Despite HLA matching, graft rejection due to mismatched mHA remains a major obstacle of this curative modality. The mHA are HLA-bound peptides derived from normal protein polymorphisms in the coding sequences of their homologous genes, which may differ between graft donor and recipient. In turn, these differences may affect recipient/donor peptide processing/MHC binding or T-cell recognition of the peptide/MHC complex.¹

Identifying gene polymorphisms leading to the generation of minor HA is of utmost importance and may lead to novel strategies of improving HSCT outcome, as they have been implicated in GVHD and in GVL effect. In this study, data are presented suggesting the involvement of glutathione S-transferase theta-1 (GSTT1) mismatch in Ab-mediated rejection of HSCT in children with congenital hemoglobinopathies (CHs).

GSTT1 is a phase II detoxifying enzyme encoded by *GSTT1*, which often features a deletion polymorphism variant.² Homozygous deletion of this gene (GSTT1 null) occurs in 20% of the Caucasian population, 25% of African Americans and 50% of Asians and results in a nonconjugator phenotype of GSTT1.³

The importance of GSTT1 genotype mismatch between donor and recipient in the setting of liver and renal transplantation has been reported. In liver transplantation, it may lead to *de novo* hepatitis,⁴ whereas in kidney transplantation, chronic allograft rejection has been demonstrated.⁵

In this study on the GSTT1 genotype in patients undergoing HSCT for the treatment of CH, it became apparent that the frequency of graft rejection among patients with a GSTT1-null genotype was significantly greater than in patients who were GSTT1 positive. Anti-GSTT1 antibodies were demonstrated in the sera of GSTT1-null patients who experienced graft rejection, suggesting that GSTT1 mismatch between donor and recipient may result in an Ab-mediated immune response against GSTT1 displayed on transplanted stem cells in recipients with a GSTT1-null genotype.

GSTT1 genotype, presence of anti-GSTT1 antibodies, HSCT outcome and relevant clinical data of 18 CH patients who underwent HSCT are presented herein.

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Patients and methods

Patients

A total of 18 patients underwent allogeneic HSCT because of CH (Tables 1 and 2). Median age at transplantation was 5.5 years (range: 10 months–10 years). All patients received a myeloablative conditioning regimen based on BU and CY (Tables 1 and 2). Five patients with class I thalassemia received BU, CY, anti-thymocyte globulin, fludarabine and moderate T-cell depletion, as described elsewhere.⁶ Six patients with class III thalassemia received protocol P26⁷ using hydroxyurea and azathioprine and later, fludarabine, CY and BU. GVHD prophylaxis, the number of CD34+ cells per kg infused and the blood groups of donor and recipient are included in Tables 1 and 2. GSTT1 genetic analysis and incidence of graft rejection were evaluated for all patients (Tables 1 and 2). Serum samples for anti-GSTT1 Ab determination were available in 14 patients (Tables 1 and 2).

Parents or guardians of participants signed informed consent according to a protocol reviewed and approved by the National Committee for Genetic Studies and by the local institutional review board.

GSTT1 genotyping

Pretransplant peripheral blood samples were collected from the 18 patients described above and genomic DNA was

extracted using the Qiamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

Homozygous deletion of the *GSTT1* gene was determined by PCR using Thermo-Start PCR master mix (Abgene, Epsom, UK) and the following primers: 5'-TCTTTTGCA TAGAGACCATGACCAG-3' and 5'-CTCCCTACTCCA GTAACCTCCGACT-3'.⁸ Control PCRs were carried out with primers encoding a segment of the thiopurine *S*-methyltransferase gene (5'-CCACCATACCCAGCTCATTT-3' and 5'-CCTCAAAAACATGTGAGTGTGA-3'). PCR products were separated on 1.5% agarose TAE gels.

Anti-GSTT1 Ab determination

Serum samples were assayed for the presence of anti-GSTT1 antibodies by ELISA, using human recombinant GSTT1 protein according to the manufacturer's protocol (human anti-GSTT1 ELISA kit, Biomedical Diagnostics, Seville, Spain). Briefly, diluted samples and provided positive and negative controls (in duplicate) were incubated with immobilized GSTT1 protein in a microwell plate. The unbound sample was washed away and alkaline phosphatase-labeled anti-human IgG was added. After additional washes, a chromogenic substrate was added and color intensity was measured at 405 nm. According to the manufacturer, values ≤ 0.5 were considered as negative for GSTT1 antibodies (or below the assay's detection limit)

Table 1 Demographics of patients with GSTT1-positive genotype

Age	Diagnosis	Graft/source	CD34 ¹⁰ /kg	Conditioning/ regimen	GVHD/ prophylaxis	Blood group donor/recipient	Anti- GSTT1/Ab	Rejection
1 2 y	Thal. major	PB/MSD	10.7	BU, CY, ATG, Flu	TCD/10 ⁵ per kg	B +/O+	ND	No
2 10 y	Thal. major	PB/MSD	9.1	BU, CY	CsA/MTX	O +/O+	Negative	No
3 10 y	Thal. major	PB/MSD	9.1	P26	CsA/MTX	AB +/AB+	ND	No
4 10 y	Thal. major	PB/MSD	9.2	P26	CsA/MTX	A +/A+	Negative	No
5 3.5 y	Thal. major	PB/MSD	20.9	P26	CsA/MTX	O +/O+	Negative	No
6 9 y	Thal. major	PB/MSD	6.5	P26	CsA/MTX	A +/A+	Negative	No
7 5 y	Sickle cell anemia	PB/MSD	20	BU, CY, ATG, Flu	CsA/MTX	O +/O+	Negative	No
8 4 y	Thal. major	MUD/PB	14.6	BU, CY, ATG, TT	CsA/MTX	O +/A+	Negative	No
9 2 y	Thal. major	MSD/PB	10	BU, CY, ATG, Flu	TCD/10 ⁵ per kg	A +/A+	ND	No
10 6 y	Sickle thal.	MSD/PB+CB	12.4	P26	CsA/MTX	B +/B+	Negative	No
11 10 months	Thal. major	MSD/PB	8	BU, CY, ATG, Flu	TCD/10 ⁵ per kg	B +/B+	ND	No

Abbreviations: ATG = anti-thymocyte globulin; CB = cord blood; Flu = fludarabine; GSTT1 = glutathione *S*-transferase theta-1; MSD = matched sibling donor; MUD = matched unrelated donor; ND = no data.; PB = peripheral blood; TCD = T-cell depletion; Thal = thalassemia; TT = thiotepa; y = years.

Table 2 Demographics of patients with GSTT1-null genotype

Age (years)	Diagnosis	Graft/ source	CD34 (10 ⁶ per kg)	Conditioning/ regimen	GVHD/ prophylaxis	Blood group donor/ recipient	Anti-GSTT1/ Ab	Rejection/ (months after transplant)	RBC transfusions(n)
1 4	Thal. major	MSD/PB	8.1	BU, CY, ATG, Flu	TCD/10 ⁵ per kg	AB-/AB-	Positive/>1	No	71
2 10	Sickle cell anemia	MSD/PB	7.1	P26	CsA/MTX	A +/O-	Positive/>1	Yes (+16)	121
3 1	Thal. major	MSD/PB	9.7	BU, CY, ATG, Flu	TCD/10 ⁵ per kg	O +/O+	Negative	No	10
4 2	Thal. major	Unrelated/CB	6 × 10 ⁵	BU, CY, ATG	CsA	O +/B+	Intermediate/ >0.94	Yes (+1)	28
5 8	Thal. major	MSD/BM	1.7	P26	CsA, CY, MTX	A +/B+	Negative	Yes (+8)	90
6 10	Thal. major	MSD/BM	5.6	P26	CsA, CY, MTX	B -/B-	Positive/>1	Yes (+8)	105
7 8	Thal. major	MSD	ND	BU, CY	ND	O +/O+	Positive/>1	Yes (+2)	129

Abbreviations: ATG = anti-thymocyte globulin; CB, cord blood; Flu = fludarabine; GSTT1 = glutathione *S*-transferase theta-1; MSD = matched sibling donor; ND = no data.; PB = peripheral blood; TCD = T-cell depletion; Thal. = thalassemia.

and values ≥ 1 were considered as positive for GSTT1 antibodies. All serum samples were taken before SCT, except those of patients 5, 6 and 7 in Table 2, which were taken after the occurrence of rejection.

Results

In the presented cohort, comprising 18 patients who underwent HSCT due to CH, seven patients had a GSTT1-null genotype, five of whom experienced graft failure at several time points after transplantation. Patient 2 rejected the graft 16 months after transplantation after stopping CsA. Patient 4 rejected the graft early and, by 1 month of transplant, there was no evidence of donor cells. Patients 5 and 6 rejected the graft 8 months after transplantation during CsA tapering off. Patient 7 rejected the graft 2 months after transplant (Table 2). GSTT1 antibodies were demonstrated in the serum of four of these patients (Table 2). The fifth patient experienced rejection likely due to low CD34+ cells in the graft (1.7×10^6 per kg), as no antibodies against GSTT1 were demonstrated. Interestingly, one patient with a GSTT1-null genotype (patient 1, Table 2) had anti GSTT1 antibodies in her serum, yet no rejection occurred. She received 71 RBC transfusions, explaining the presence of antibodies in her serum. She did not reject the graft probably because her donor had a GSTT1-null genotype as well.

Among the seven patients with a GSTT1-null genotype (Table 2), the median age of the five patients who rejected the graft was 8 years, with a median number of 105 RBC transfusions given, whereas the two patients who did not experience rejection were aged 1 year and 4 years and received 10 and 71 RBC transfusions, respectively.

The donor DNA of patient 7 in Table 2 was genotyped and found to be GSTT1 positive, thus completing the picture of mismatched GSTT1 (null in the recipient, positive in the donor), appearance of GSTT1 antibodies and, ultimately, graft rejection. Unfortunately, this study did not have the institutional review board approval for GSTT1 genotyping of all the donors' DNA.

No graft failure occurred in the 11 patients with a GSTT1-positive genotype (Table 1). Accordingly, no antibodies were found in the sera of seven of those patients.

Discussion

In this study, data on GSTT1 genotype, presence of anti-GSTT1 antibodies, HSCT outcome and relevant clinical data of 18 CH patients who underwent HSCT are presented.

Of the 18 children included in this study, 7 had a GSTT1-null genotype (Table 2). Of them, five (71.4%) experienced graft failure at various time points after transplantation (1–16 months after transplantation), four of whom displayed anti-GSTT1 antibodies. Three had high levels of Ab and one had an intermediate level (as defined by the GSTT1 Ab kit manufacturer, see Patients and methods).

No anti-GSTT1 Ab or cases of graft rejection were detected in patients with a GSTT1-positive genotype (Table 1).

Thus, the data suggest that in patients with a GSTT1-null genotype who receive a GSTT1-positive graft, an Ab-mediated immune response against GSTT1 displayed on transplanted stem cells may occur and result in graft rejection.

GSTT1 mismatch between transplant donor and recipient has previously been implicated in post-liver and -kidney transplant complications. In liver transplant patients, GSTT1 has been identified as a potential alloantigen-mediated autoimmune hepatitis (IH), referred to as *de novo* IH, when the liver recipients were homozygous null for the gene, but received a GSTT1-positive graft.^{4,9} The increased risk of *de novo* IH in this group was statistically significant, promoting GSTT1 genotyping of liver transplant donors and recipients as a predictive marker for the development of *de novo* IH.⁹

In GSTT1-null kidney transplant patients with a GSTT1-positive donor, an association between chronic humoral kidney rejection and post-transplant production of anti-GSTT1 antibodies has been demonstrated.¹⁰ Renal biopsies of four patients with a GSTT1 donor/recipient mismatch, who developed anti-GSTT1 antibodies, showed pathological lesions compatible with chronic Ab-mediated rejection. In three of these patients, C4d+ staining in peritubular capillaries was also found.

Interestingly, no donor-specific anti-HLA antibodies were detected in any of these four patients, suggesting a role for GSTT1 in anti-graft immune response.⁵ These findings were confirmed by Alvarez-Marquez *et al.*¹⁰ who showed that in eight renal transplants performed in the context of a GSTT1-positive donor/GSTT1-negative recipient, which resulted in graft dysfunction and CD4+ deposition in the kidney, 75% of the recipients developed anti-GSTT1 antibodies. In three of these, no other donor-specific HLA I/II or MHC class I chain-related gene A (MICA) antibodies were detected.

Anti-GSTT1 antibodies have been documented in GSTT1-null individuals with a history of blood transfusions or a previous pregnancy,¹¹ because of GSTT1-positive transfusions or pregnancy of a GSTT1-positive fetus, which could induce production of anti-GSTT1 antibodies in a GSTT1-negative individual.

In a study on GST enzyme expression in hematopoietic cell lines, it was demonstrated that expression of GST isoenzymes shows distinct lineage differences.¹² GSTT1 gene expression and protein were found predominantly in erythroid cell lines. GSTT1 was not expressed in either lymphoid cell lines or in three myeloid cell lines. This is compatible with its abundant expression described in erythrocytes.¹³ Those facts may consolidate the theory on the occurrence of immune response and Ab production in a situation in which repeated RBC transfusion is given to a patient harboring a null GSTT1 genotype.

GSTT1-null patients diagnosed with thalassemia major (83% of the present cohort) undergo multiple RBC transfusions over a period of years. Because, similar to Caucasian populations, >80% of the Israeli population carries at least one allele of GSTT1 (data not shown), the likelihood of exposure to this Ag through transfusion is very high and could result in anti-GSTT1 Ab production in the GSTT1-null recipient, as previously described.¹¹

A similar scenario is feasible in patients suffering from a variety of CHs who receive blood transfusions regularly and may be candidates for HSCT. Interestingly, Lucarelli *et al.*¹⁴ have shown that a major complication of HSCT in class III thalassemic patients after multiple blood transfusions and development of liver fibrosis was graft rejection at an incidence rate of 30%. This incidence of rejection was dramatically reduced by a prolonged (up to 45 days) immunosuppressive regimen that included azathioprine and hydroxyurea.⁷ Using this regimen with the addition of fludarabine to BU and CY brought down the incidence of rejection to 8%.⁷ The likelihood of rejection was shown to be inversely related to the transfusion burden in these patients. The 5-year probabilities of rejection were 53% and 24% in patients who received more or less than 100 RBC transfusions before transplantation, respectively.¹⁴

Taken together, the findings of this study may indicate that anti-GSTT1 antibodies in patients with a GSTT1-null genotype are predictive of HSCT rejection in the event of a GSTT1-positive donor. Absence of anti-GSTT1 antibodies before transplantation predicts nonrejection of HSCT with a specificity of nearly 90%. Presence of antibodies without genotyping the graft donor or recipient predicts HSCT rejection with a sensitivity of 80%. This sensitivity can be increased if in addition to examining the recipient anti-GSTT1 Ab level, donor DNA is genotyped and found to be GSTT1 positive.

This study has several limitations. The first limitation is the timing of GSTT1 Ab analysis. As this was a retrospective study, for some patients, pretransplant serum was unavailable (patients 5, 6 and 7 on Table 2), making it impossible to establish whether anti-GSTT1 Ab production was triggered by the GSTT1-mismatched graft or by mismatched RBC transfusions given before transplantation. In addition, the relationship between Ab-titer appearance and timing of graft rejection could not be established. Another limitation is the small number of patients included, although they all shared a similar diagnosis.

To consolidate the above results, a larger cohort of patients is needed and the study should be carried out in a prospective manner, whereby donor and recipient GSTT1 genotypes are analyzed before transplantation and anti-GSTT1 Ab levels are measured before and at several time points after transplantation.

On the basis of the presented data, it is proposed that GSTT1 may function as a minor HA and that mismatch of this locus has a role in hematopoietic graft rejection.

Conflict of interest

The authors declare no conflict of interest.

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References

- Murata M, Warren EH, Riddell SR. A human minor histocompatibility antigen resulting from differential expression due to a gene deletion. *J Exp Med* 2003; **197**: 1279–1289.
- Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM *et al*. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. *Biochem J* 1994; **300**(Part 1): 271–276.
- Landi S. Mammalian class theta GST and differential susceptibility to carcinogens: a review. *Mutat Res* 2000; **463**: 247–283.
- Yoshizawa K, Shirakawa H, Ichijo T, Umemura T, Tanaka E, Kiyosawa K *et al*. *De novo* autoimmune hepatitis following living-donor liver transplantation for primary biliary cirrhosis. *Clin Transplant* 2008; **22**: 385–390.
- Aguilera I, Alvarez-Marquez A, Gentil MA, Fernandez-Alonso J, Fijo J, Saez C *et al*. Anti-glutathione S-transferase T1 antibody-mediated rejection in C4d-positive renal allograft recipients. *Nephrol Dial Transplant* 2008; **23**: 2393–2398.
- Elhasid R, Arush MB, Zaidman I, Leiba R, Barak AB, Postovsky S *et al*. Safe and efficacious allogeneic bone marrow transplantation for nonmalignant disorders using partial T cell depletion and no posttransplantation graft-versus-host-disease prophylaxis. *Biol Blood Marrow Transplant* 2007; **13**: 329–338.
- Sodani P, Gaziev D, Polchi P, Erer B, Giardini C, Angelucci E *et al*. New approach for bone marrow transplantation in patients with class 3 thalassemia aged younger than 17 years. *Blood* 2004; **104**: 1201–1203.
- Buchard A, Sanchez JJ, Dalhoff K, Morling N. Multiplex PCR detection of GSTM1, GSTT1, and GSTP1 gene variants: simultaneously detecting GSTM1 and GSTT1 gene copy number and the allelic status of the GSTP1 Ile105Val genetic variant. *J Mol Diagn* 2007; **9**: 612–617.
- Aguilera I, Sousa JM, Gavilan F, Bernardos A, Wichmann I, Nunez-Roldan A. Glutathione S-transferase T1 mismatch constitutes a risk factor for *de novo* immune hepatitis after liver transplantation. *Liver Transpl* 2004; **10**: 1166–1172.
- Alvarez-Marquez A, Aguilera I, Gentil MA, Caro JL, Bernal G, Fernandez Alonso J *et al*. Donor-specific antibodies against HLA, MICA, and GSTT1 in patients with allograft rejection and C4d deposition in renal biopsies. *Transplantation* 2009; **87**: 94–99.
- Wichmann I, Aguilera I, Sousa JM, Bernardos A, Garcia Nunez EJ, Vigil E *et al*. Antibodies against glutathione S-transferase T1 in non-solid organ transplanted patients. *Transfusion* 2006; **46**: 1505–1509.
- Wang L, Groves MJ, Hepburn MD, Bowen DT. Glutathione S-transferase enzyme expression in hematopoietic cell lines implies a differential protective role for T1 and A1 isoenzymes in erythroid and for M1 in lymphoid lineages. *Haematologica* 2000; **85**: 573–579.
- Schroder KR, Hallier E, Meyer DJ, Wiebel FA, Muller AM, Bolt HM. Purification and characterization of a new glutathione S-transferase, class theta, from human erythrocytes. *Arch Toxicol* 1996; **70**: 559–566.
- Lucarelli G, Clift RA, Galimberti M, Polchi P, Angelucci E, Baronciani D *et al*. Marrow transplantation for patients with thalassemia: results in class 3 patients. *Blood* 1996; **87**: 2082–2088.