Human Immunology 70 (2009) 457-460

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Possible impact of *MADCAM1* gene single nucleotide polymorphisms to the outcome of allogeneic hematopoietic stem cell transplantation

Zuzana Ambruzova [a], Frantisek Mrazek [a], Ludek Raida [b], Anna Stahelova [a], Edgar Faber [b], Karel Indrak [b], Martin Petrek [a,*]

[a] *Laboratory of Immunogenomics and Proteomics, Department of Immunology, Palacky University and University Hospital Olomouc, Czech Republic*
[b] *Department of Hematooncology, Palacky University and University Hospital Olomouc, Czech Republic*

ARTICLE INFO

Article history:
Received 18 September 2008
Accepted 2 March 2009
Available online 12 March 2009

Keywords:
Graft-versus-host disease
Hematopoietic stem cell transplantation
MAdCAM-1
Single nucleotide polymorphism
Overall survival

ABSTRACT

Mucosal addressin cell adhesion molecule–1 (MAdCAM-1) contributes to the recruitment of donor T cells into the mucosal tissues of the recipient after allogeneic hematopoietic stem cell transplantation (aHSCT). The aim of our study was to determine whether selected single nucleotide polymorphisms (SNPs) of the *MADCAM1* gene are associated with development of serious complications after aHSCT. Three *MADCAM1* gene single nucleotide polymorphisms (rs758502 C/T, rs2302217 A/G, rs3745925 G/T) were genotyped by polymerase chain reaction with sequence-specific primers in 87 Czech, HLA-identical donor-recipient aHSCT pairs. *MADCAM1* rs2302217 AA homozygous recipients developed chronic GVHD more frequently than patients with other genotypes (65% vs. 34%; $p = 0.025$). Furthermore, multivariate analysis revealed the *MADCAM1* rs2302217 AA genotype in recipient being also an independent factor associated with development of acute GVHD ($p = 0.036$) and decreased overall survival ($p = 0.001$). These data suggest that *MADCAM1* gene polymorphisms may be associated with the risk of chronic GVHD and may, also, affect mortality related to aHSCT.

1. Introduction

Although allogeneic hematopoietic stem cell transplantation (aHSCT) has become a routine means of curative therapy for many hematologic malignant and nonmalignant diseases, its outcome is affected by development of different serious post-transplantation complications such as graft-versus-host disease (GVHD), infection, and relapse. Various non– human leukocyte antigen (HLA) factors, including genetic polymorphisms of cytokines, their receptors and other molecules with immune functions are intensively studied for their possible relevance for aHSCT outcome [1–3].

GVHD is one of the most important complications that influence morbidity and mortality after the aHSCT. The main effector cells in GVHD are the donor T cells, which exit lymphoid tissues during the activation phase of GVHD and migrate to mucosal sites and parenchymal GVHD target organs, where they cause tissue damage. The molecular interactions necessary for migration and distribution of activated donor T cells in the host tissues have recently been studied, especially for their therapeutic potential (4).

Adhesion molecules, in cooperation with chemokines, drive organ-specific homing of donor lymphocytes to either peripheral or mucosal lymph nodes (5) via interaction with their counterparts expressed on lymphocytes. Mucosal addressin cell adhesion molecule–1 (MAdCAM-1) is an endothelial cell marker that is recognized by its ligands on lymphocytes: L-selectin and $\alpha_4\beta_7$ integrin [6]. MAdCAM-1 is highly expressed on intestinal endothelium and has, recently, been shown to play an important role in the development of acute GVHD in animal model [7,8]. It has been speculated that particular variants of the *MADCAM1* gene described previously [9] may affect structure and/or expression of MAdCAM-1 molecule in the recipient of the allogeneic hematopoietic stem cell graft and therefore may also influence aHSCT outcomes. In this preliminary study of 87 hematologic patients who underwent allogeneic HSCT from their HLA identical donor, we therefore investigated a possible association of three selected single nucleotide polymorphisms spanning the *MADCAM1* gene (rs758502 C/T, rs2302217 A/G, rs3745925 G/T) with the development of acute or chronic GVHD, transplant-related mortality, and overall survival after aHSCT.

2. Subjects and methods

2.1. Patients and donors

A total of 87 patients were enrolled (37 female and 50 male; age range 18 – 61 years, median 43 years) after aHSCT at the Department of Hematooncology of University Hospital Olomouc. Also enrolled were 87 HLA-matched related ($n = 70$) and unrelated ($n = 17$) donors (34 female and 53 male; age range 18 – 69 years, median 40 years). Patients were referred for aHSCT mainly because of

* Corresponding author.
E-mail address: martin.petrek@fnol.cz (M. Petrek).

0198-8859/09/$32.00 - see front matter © 2009 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.humimm.2009.03.008

hematologic malignancy (94.3%); 5 patients had sideropenic aplastic anemia. Acute leukemia (34 acute myeloid leukemia patients and nine acute lymphocytic leukemia patients), non-Hodgkin lymphoma (14 patients), and chronic myeloid leukemia (10 patients) were the most common underlying diagnoses. The level of HLA identity was established according the European Federation for Immunogenetics (EFI) standards. In cases of related aHSCT pairs, all donors were genotypically identical (HLA-A,-B-DR loci as minimum); in cases of unrelated aHSCT pairs, only those with complete identity at allelic ("high-resolution") level of HLA-A,-B,-Cw,-DRB1. and -DQB1 loci were enrolled. 48 patients (55%) received a non-myeloablative conditioning regimen. Acute GVHD was diagnosed in 33 patients (38%), and 3 patients who died before day +30 post-transplantation without significant aGVHD symptoms were excluded from aGVHD association analysis. Acute GVHD grade II was observed in 23 patients and severe aGVHD grade III and IV in 10 cases (4 and 6, respectively). Chronic GVHD was diagnosed in 31 recipients (43%); 15 patients who died before day +100 after aHSCT and were not evaluable for cGVHD were excluded from the cGVHD association analysis. Fifteen out of 47 patients who did not survive after aHSCT died from progression of the underlying disease.

The study was approved by the Ethics Committee of the Medical Faculty Palacky University and Faculty Hospital Olomouc. All subjects signed the informed consent about use of DNA samples, taken primarily for diagnostic purposes (HLA genotyping), also about the research purposes of this study.

2.2. Genetic analysis

DNA was extracted from the peripheral blood of the patients and their donors by the standard salting out method and was stored at −20°C. Three *MADCAM1* gene SNPs were selected from those investigated previously by Bowlus *et al.* (9) to cover different regions (coding and regulatory) of the *MADCAM1* gene. This selection of *MADCAM1* SNPs was further motivated by the possibility to compare the data with the frequencies observed in another Caucasoid population [9].

Genotypes for three investigated SNPs spanning the *MADCAM1* gene were determined using polymerase chain reaction (PCR) with the following sequence-specific primers (SSPs). *MADCAM1* (rs758502 C/T, 5′ flanking region)–specific primers: 5′AGG CCG CCC ACC TAG CAG/A, constant primer: 5′TAA ACA AGC CAC CAG CGG AG. *MADCAM1* (rs2302217 A/G, Exon 3, synonymous)–specific primers: 5′CAG CTG ACC GTC TCC CCA/G, constant primer: 5′GCT CTG TCA CCC TGA ACA G. *MADCAM1* (rs3745925 G/T, Exon 4, coding)–specific primers: 5′AGA TCT CCC AGG CTG GGC C/A, constant primer: 5′CAG TTA AGA GAA GCC CAC TG. Reaction conditions and internal control primers were adopted from phototyping methodology [10].

2.3. Statistical analysis

The genotypes for *MADCAM1* SNPs were calculated by direct counting, and appropriate allele and phenotype (carriage rates) frequencies were calculated. Distribution of genotypes was tested for conformity with the Hardy-Weinberg equilibrium using the χ^2 test. *MADCAM1* haplotype analysis was performed using software Arlequin 3.0 (University of Berne, Switzerland; http://cmpg.unibe.ch/software/arlequin3) [11]. Associations between patient or donor *MADCAM1* genotypes/alleles and main outcome of the study (*i.e.,* acute or chronic GVHD) were analysed using the SPSS 15.0 software (SPSS Inc, Chicago, IL). Bonferroni correction [12] was used to provide adjustment for multiple comparisons. For univariate analysis Pearson's χ^2 test was used, and the multivariate analysis was performed using binary logistic regression models. Factors biologically considerable in the occurrence of GVHD were included the following: diagnosis (malignancy vs. non-malignancy), stem cell source, conditioning regimen, GVHD prophylaxis, donor–recipient gender combination (female donor in male recipients vs. others), and *MADCAM1* variants. The Kaplan-Meier and Cox regression analysis for overall survival and transplant-related mortality was performed using SPSS software (version 15.0). In addition to factors mentioned above, the Cox regression analysis of overall survival also included acute and chronic GVHD data as covariates. Transplant-related mortality was defined as death from any cause other than progression of the underlying disease during the course of treatment. Values of $p < 0.05$ were considered statistically significant and values between 0.05 and 0.1 as indicative of a trend.

3. Results

3.1. Distribution of MADCAM1 SNPs in study population

Distributions of *MADCAM1* genotypes for each of three SNPs in the groups of patients after HSCT and their donors were in compliance with Hardy-Weinberg equilibrium except of *MADCAM1* rs3745925 genotype frequency in donors ($p = 0.030$, $p_{corr} > 0.050$). No significant difference in the proportion of *MADCAM1* alleles/genotypes was observed between the groups of patients and donors (Table 1). *MADCAM1* genotype frequencies in patients and their donors were comparable with frequencies of *MADCAM1* genotypes reported in a Scandinavian healthy control group [9] except for higher frequency of GG genotype and lower frequency of GT genotype (rs3745925) in our group of donors ($p = 0.050$).

3.2. Analysis of MADCAM1 SNPs association with development of GVHD

To determine possible associations between *MADCAM1* polymorphisms and GVHD, we compared proportions of particular *MADCAM1* alleles and genotypes between the recipients with and without clinically significant acute (grades II–IV) or chronic GVHD (Table 2). Although only a trend for more frequent development of acute GVHD in *MADCAM1* rs2302217 AA homozygous patients ($p = 0.090$) was observed in univariate analysis, this association was more apparent in multivariate analysis. Regression model controlling for other relevant factors (see Subjects and methods section) identified the *MADCAM1* AA genotype in recipient as independent factor associated with development of acute GVHD ($p = 0.036$; OR = 3.4; 95% CI = 1.1–10.7). In the context of chronic GVHD, recipients homozygous for A allele of the *MADCAM1* rs2302217 SNP developed chronic GVHD more frequently than patients with other genotypes (Table 2). Chronic GVHD was present in 11 out of 17 patients with AA genotype (64.7%) compared with 18 out of 53

Table 1
Genotype frequencies of the investigated *MADCAM1* gene SNPs in patients and donors

	Patients $N = 87$	Donors $N = 85$
MADCAM1 T/C (rs758502)		
Genotype CC	0.43 (37)	0.53 (44)
Genotype TC	0.47 (41)	0.37 (31)
Genotype TT	0.10 (9)	0.10 (8)
MADCAM1 G/A (rs2302217)		
Genotype AA	0.26 (22)	0.35 (29)
Genotype GA	0.51 (43)	0.40 (33)
Genotype GG	0.24 (20)	0.24 (20)
MADCAM1 G/T (rs3745925)		
Genotype GG	0.64 (54)	0.77 (63)[a]
Genotype GT	0.33 (28)	0.18 (15)
Genotype TT	0.04 (3)	0.05 (4)

Data are presented as relative proportions of genotypes with the absolute numbers in parentheses. Distribution of genotypes in particular groups was in compliance with Hardy-Weinberg equilibrium, except of SNP G/T in group of donors ($p = 0.030$, $p_{corr} > 0.050$).

[a] *MADCAM1* (rs3745925) GG genotype frequency: $p = 0.060$ for comparison patients with donors.

Table 2
Genotype frequencies of the investigated *MADCAM1* gene SNPs in the groups of Czech patients with (+)/without (−) acute and chronic GVHD

	Patient group[a]					
	aGVHD+	aGVHD−	p^b	cGVHD+	cGVHD−	p^b
MADCAM1 T/C (rs758502)	$n = 35$	$n = 49$		$n = 31$	$n = 41$	
Genotype CC	0.37 (13)	0.45 (22)	0.477	0.52 (16)	0.32 (13)	0.088
Genotype TC	0.54 (19)	0.43 (21)		0.35 (11)	0.59 (24)	
Genotype TT	0.09 (3)	0.12 (6)	0.592	0.13 (4)	0.10 (4)	0.674
MADCAM1 G/A (rs2302217)	$n = 34$	$n = 48$		$n = 29$	$n = 41$	
Genotype AA	0.15 (5)	0.31 (15)	0.086	0.38 (11)	0.15 (6)	**0.025**
Genotype GA	0.62 (21)	0.46 (22)		0.34 (10)	0.63 (26)	
Genotype GG	0.23 (8)	0.23 (11)	0.948	0.28 (8)	0.22 (9)	0.588
MADCAM1 G/T (rs3745925)	$n = 34$	$n = 48$		$n = 29$	$n = 41$	
Genotype GG	0.59 (20)	0.69 (33)	0.354	0.76 (22)	0.54 (22)	0.058
Genotype GT	0.35 (12)	0.29 (14)		0.21 (6)	0.44 (18)	
Genotype TT	0.06 (2)	0.02 (1)	0.367	0.03 (1)	0.02 (1)	0.803

Data are presented as relative proportions of genotypes, with the absolute numbers in parentheses.
[a]Three patients (with regard to the acute GVHD) and 15 patients (for chronic GVHD) had to be excluded from the analysis (see Subjects and methods section).
[b]*p* Values for comparison of particular homozygous genotype (versus carriage of an alternative allele) between GVHD+ and GVHD− patients.

recipients with other genotypes (34.0%; $p = 0.025$). However, when strict correction for multiple comparisons was performed, these associations did not attain significance ($p_{corr} > 0.050$). Except for the *MADCAM1* rs2302217 AA genotype described above, no association between other *MADCAM1* gene variants (Table 2) or haplotypes estimated by software Arlequin 3.0 (data not shown) in recipients and donors and in the development of GVHD was found.

3.3. *Analysis of* MADCAM1 *SNPs association with survival after aHSCT*

Apart from investigating the association of selected SNPs with GVHD, we were also interested to determine whether these polymorphisms might be relevant for survival after aHSCT. In case of overall survival, Cox regression model was adjusted for all considerable clinical and biologic (genetic) factors available for analysis (diagnosis, donor relationship, donor–recipient gender combination, conditioning regimen, stem-cell source, GVHD prophylaxis, and MADCAM1 SNP variants), together with the presence of aGVHD and/or cGVHD in recipients. We observed the presence of acute ($p = 1*10^{-5}$; hazard ratio [HR] = 4.352; 95% CI = 2.287–8.281) and chronic ($p < 5*10^{-5}$; HR = 5.700; 95% CI = 2.526–12.873) GVHD and the *MADCAM1* rs2302217 AA genotype ($p = 0.001$; HR = 2.999; 95% CI = 1.524–5.902) as independent factors associated with decrease of overall survival. Survival analysis did not identify any association between *MADCAM1* genotype frequencies and transplant-related mortality after aHSCT.

4. Discussion

4.1. *Relevance of* MADCAM1 *gene polymorphism in aHSCT*

Serious complications that follow the allogeneic hematopoietic stem cell transplantation in 30–50% of cases remain the leading cause of its morbidity and mortality. A wide spectrum of non-HLA genes related to patients and donors have recently been investigated for their possible role in the development of GVHD, infections, and their impact on transplant-related mortality and overall survival [13,14]. The present study describes an association of *MADCAM1* genetic polymorphisms with development of chronic GVHD and overall survival.

The potential importance of *MADCAM1* gene and its variants in aHSCT has been supported by animal models of GVHD [7,8]. We therefore selected *MADCAM1* gene as a candidate for implication in the development aHSCT complications. By analogy with its suggested role in GVHD, studies of the MAdCAM-1 molecule and its gene variants' participation in the recruitment of T-lymphocytes to the sites of ongoing inflammation have been performed in a spectrum of gastrointestinal diseases mainly of inflammatory nature [9,15,16]. However, to the best of our knowledge, no study investigating the relationship between *MADCAM1* gene polymorphism and aHSCT outcome has yet been published.

Three SNPs of *MADCAM1* gene—one from 5′ flanking region (rs758502) and two exonic (rs2302217, synonymous and rs3745925, coding)—have been investigated in this study. Except for the fact that these SNPs span various regions within the *MADCAM1* gene (with priority for coding and promoter), their selection was convenient also from the methodologic point of view, as the distribution of these variants has already been reported in other populations [9]. It must be acknowledged, however, that the plausible effects of these SNPs on MAdCAM-1 expression and signaling have not yet been described. Here the interpretation of our data in the context of aHSCT remains at the level of speculation: particular *MADCAM1* gene variants may affect structure and/or expression of MAdCAM-1 molecule and in consequence may influence also affinity of donor T lymphocytes to the mucosal and lymphoid tissues of the recipient. In this context, genetically determined long-term deregulation of MAdCAM-1 signaling may predispose individuals carrying particular *MADCAM1* variants (haplotypes) to the development of chronic GVHD.

4.2. *Study limitations*

The number of patients included in our study is suboptimal for genetic association study and is not completely homogenous regarding some clinical parameters (*e.g.,* diagnosis, related/unrelated aHSCT, conditioning regimens). Therefore, the effect of *MADCAM1* variants in specific clinical conditions may be confounded (*i.e.,* masked). Nevertheless, the results of multivariate analyses controlling for the majority of considerable covariates are provided to support the discussed context of *MADCAM1* gene polymorphism. Because there are no other reports on *MADCAM1* gene variants in aHSCT, the results of this study should be considered preliminary until replicated.

4.3. *Conclusions*

In summary, the preliminary data from this study suggest that *MADCAM1* rs2302217 AA genotype in recipients may be associated with the risk of GVHD and decreased overall survival after aHSCT. Before further implications are drawn from our findings, in accordance with current rules of genetic association studies [17,18], the possible impact of *MADCAM1* SNP variants for GVHD and survival after aHSCT needs to be confirmed in substantially larger cohorts of donor-recipient aHSCT pairs in which complex effects of other clinical and genetic factors can be considered. Furthermore, the

future replication of our results may benefit from more complete coverage of the *MADCAM1* gene based on a tagging approach with Hap-Map project data. Despite its preliminary character, our report of a relationship between variation in the *MADCAM1* gene and GVHD is intended to invoke replication and verification studies.

Acknowledgments

This study was supported by the Internal Grant Agency of the Czech Ministry of Health (IGA NR9099), and in part also by the Czech Ministry of Education, Youth and Sports MSM6198959205.

References

[1] Dickinson AM. Risk assessment in hematopoietic stem cell transplantation: pre-transplant patient and donor factors: Non-HLA genetics. Best Pract Res Clin Hematol 2007;20:189–207.
[2] Dickinson AM, Charron D. Non-HLA immunogenetics in hematopoietic stem cell transplantation. Curr Opin Immunol 2005;17:517–25.
[3] Mullally A, Ritz J. Beyond HLA: The significance of genomic variation for allogeneic hematopoietic stem cell transplantation. Blood 2007;109:1355–62.
[4] Wysocki CA, Panoskaltsis-Mortari A, Blazar BR, Serody JS. Leukocyte migration and graft-vesus-host disease. Blood 2005;105:4191–9.
[5] Ager A. Lymphocyte recirculation and homing: Roles of adhesion molecules and chemoattractants. Trends Cell Biol 1994;4:326–32.
[6] Elangbam CS, Qualls CW, Jr., Dahlgren RR. Cell adhesion molecules—update. Vet Pathol 1997;34:61–73.
[7] Ueha S, Murai M, Yoneyama H, Kitabatake M, Imai T, Shimaoka T, et al. Intervention of MAdCAM-1 or fractalkine alleviates graft-versus-host reaction associated intestinal injury while preserving graft-versus-tumor effects. J Leukoc Biol 2007;81:176–85.
[8] Murai M, Yoneyama H, Ezaki T, Suematsu M, Terashima Y, Harada A, et al. Peyer's patch is the essential site in initiating murine acute and lethal graft-versus-host reaction. Nat Immunol 2003;4:154–60.
[9] Bowlus CL, Karlsen TH, Broomé U, Thorsby E, Vatn M, et al. Analysis of MAdCAM-1 and ICAM-1 polymorphisms in 365 Scandinavian patients with primary sclerosing cholangitis. J Hepatol 2006;45:704–10.
[10] Bunce M, O'Neill CM, Barnardo MC, Krausa P, Browning MJ, Morris PJ, et al. Phototyping: Comprehensive DNA typing for HLA-A, B, C, DRB3, DRB4, DRB5, & DQB1 by PCR with 144 primer mixes utilising sequence-specific primers (PCR-SSP). Tissue Antigens 1995;46:355–67.
[11] Excoffier L, Laval G, Schneider S. Arlequin version 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 2005;1:47–50.
[12] Svejgaard A, Ryder LP. HLA and disease associations: Detecting the strongest association. Tissue Antigens 1994;43:18–27.
[13] Dickinson AM, Middleton PG, Rocha V, Gluckman E, Holler E & Eurobank members. Genetic polymorphisms predicting the outcome of bone marrow transplants. Br J Hematol 2004;127:479–90.
[14] Mullighan C, Heatley S, Doherty K, Szabo F, Grigg A, Hughes T, et al. Non-HLA immunogenetic polymorphisms and the risk of complications after allogeneic hemopoietic stem-cell transplantation. Transplantation 2004;77:587–96.
[15] Eksteen B, Miles AE, Grant AJ, Adams DH. Lymphocyte homing in the pathogenesis of extra-intestinal manifestations of inflammatory bowel disease. Clin Med 2004;4:173–80.
[16] Arihiro S, Ohtani H, Suzuki M, Murata M, Ejima C, Oki M, et al. Differential expression of mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in ulcerative colitis and Crohn's disease. Pathol Int 2002;52:367–74.
[17] Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, Thomas G, et al. Replicating genotype-phenotype associations. Nature 2007;447:655–60.
[18] Ioannidis JPA, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. Nat Genet 2001;29:306–9.