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HLA mismatch combinations associated with decreased risk of relapse: Implications for molecular mechanism

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Regular Article

HLA Mismatch Combinations Associated with Decreased Risk of Relapse: Implications for Molecular Mechanism.

Running Title: HLA MISMATCH COMBINATIONS RESPONSIBLE FOR GVL

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Abstract

The finding that the risk of relapse in hematological malignancy decreases after allogeneic hematopoietic stem cell transplantation (HSCT) has lead to the concept of a graft-versus-leukemia effect (GVL). This beneficial effect is considered often offset, however, by graft-versus-host disease (GVHD). Thus, improving HSCT outcomes by separating GVL from GVHD is considered a key clinical issue. This cohort study registered 4643 patients transplanted for hematological malignancy from an unrelated donor. 6 major HLA loci were retrospectively genotyped. We identified 4 HLA-Cw and 6 HLA-DPB1 mismatch combinations responsible for a decreased risk of relapse; of these, 8 of 10 combinations were different from those responsible for severe acute GVHD, including all 6 of the HLA-DPB1 combinations. Pairs with these combinations of HLA-DPB1 had a significantly improved overall survival compared to completely matched pairs. Moreover, several amino acid substitutions on specific positions responsible for a decreased risk of relapse were identified in HLA-Cw, but not in HLA-DPB1. These findings might be key to elucidating the mechanism of the decreased risk of relapse on the basis of HLA molecule. Donor selection made in consideration of these results might allow the separation of GVL from acute GVHD, especially in HLA-DPB1 mismatch combinations.

Introduction

The use of allogeneic hematopoietic stem cell transplantation (HSCT), an established treatment for hematological malignancy, is associated with several immunological events of contrary benefit to the recipient. In graft-versus-host disease (GVHD), for example, graft immune cells attack host organs, whereas in the graft-versus-leukemia effect (GVL), they eradicate residual leukemia cells [1-3]. GVL is likely to function not only in hematological malignancy but also in solid tumors, particularly breast cancer and renal cell carcinoma [4-6], in which case it is referred to as the graft-versus-tumor effect (GVT). Because both GVL and GVHD are caused by either or both major and minor histocompatibility antigen mismatches between donor and recipient, these beneficial effects of allogeneic HSCT are thought to be frequently offset by GVHD. Thus, improving HSCT outcome by separating GVL from GVHD is a key clinical issue. Importantly, however, while most such efforts have been in the area of minor histocompatibility antigen [7], few researchers have approached this problem in terms of the major histocompatibility antigen.

We recently identified 16 high-risk HLA mismatch combinations for severe acute GVHD in HLA loci. Results showed that the overall number of these high-risk mismatches was strongly associated with the occurrence of severe acute GVHD and poor overall survival (OS) [8]. We speculated that the intensity of GVL and acute GVHD in any particular mismatch might not necessarily be parallel, and that among HLA mismatch combinations not inducing severe acute GVHD, those which induce strong GVL might occur. In other words, the hypotheses of this study were that particular

mismatch combinations allow the separation of GVL from acute GVHD and that specific amino acid substitutions in HLA molecules contribute to this mechanism.

Here, as part of efforts to improve donor selection and allogeneic HSCT outcomes, we identified HLA mismatch combinations which resulted in a decreased risk of relapse in all six major HLA loci and compared them with mismatch combinations carrying a high risk of severe acute GVHD. Further, we also investigated specific amino acid substitution positions in the HLA molecule responsible for a decreased risk of relapse.

Material and Methods

Patients

This study was conducted by using clinical data that were collected prospectively at transplant centers participating in the Japan Marrow Donor Program. Patients who received a first transplant of T-cell-replete marrow for hematological malignancy from a serologically HLA-A, -B and -DR antigen-matched unrelated donor between January 1993 and December 2005 through the Japan Marrow Donor Program (n=4643) were registered. Eligible diagnoses included acute lymphoblastic leukemia (ALL); acute myeloid leukemia (AML), which included only de novo AML; chronic myeloid leukemia (CML); malignant lymphoma (ML); and multiple myeloma (MM).

Patient characteristics are shown in Table 1. Final clinical survey of the patients was completed by December 2006. Informed consent was obtained from patients and donors in accordance with the Declaration of Helsinki, and approval for the study was obtained from the Institutional Review Board of Aichi Cancer Center and the Japan Marrow Donor Program.

HLA Typing of Patients and Donors

Alleles at the HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 loci were identified by previously described methods in all 4643 pairs at Japanese Red Cross Tokyo Metropolitan Blood Center [8,9].

Matching of HLA Allele Between Patient and Donor

HLA allele mismatch among the donor-recipient pair was scored when the recipient's alleles were not shared by the donor (graft versus host vector) for all analyses.

Definition of Relapse

Relapse was defined as the recurrence of malignancy as detected by the parameter by which the malignancy was first detected, namely marrow morphology; flow cytometry; cytogenetic studies, including fluorescence in situ hybridization; electrophoresis; immunofixation assays; polymerase chain reaction-based assays for disease markers; or imaging results. The day of relapse was defined as the day on which the respective clinical, hematologic, cytogenetic or molecular relapse was recognized.

Definition of Amino Acid Substitution

Amino acid sequences of HLA-Cw and -DPB1 molecules were obtained from the IMGT/HLA sequence database (http://www.ebi.ac.uk/imgt/hla/). For example, Tyr99C-Phe99C indicated an amino acid substitution at position 99 in the HLA-C molecule in which the donor had tyrosine and the patient had phenylalanine. Substituted amino acids in HLA-Cw and -DPB1 are summarized in supplementary Tables 1 and 2.

Statistical Analysis

OS rate was assessed using the Kaplan–Meier product limit method. To eliminate the effect of competing risk, the cumulative incidence of relapse was assessed using a previously described method [10,11]. The competing event for relapse was defined as death

without relapse. Impact by the factor of interest was assessed using the log rank test. The impact of HLA allele mismatch combinations and the position and type of amino acid substitution (for example, alanine, arginine, and asparagines) of HLA molecules were evaluated using multivariable Cox regression analysis [12] for OS and the occurrence of acute GVHD, while the risk of relapse was evaluated using the multivariable proportional hazard modeling of subdistribution functions in competing risks [13].

HLA mismatch combinations were evaluated for each locus separately. When the locus of interest was evaluated, we allowed the other loci to be mismatched, with the status of such mismatches adjusted for in the same way as were other confounders. The HLA match and HLA one-allele mismatched in every locus were analyzed. For example, the A*0206-A*0201 mismatch combination meant that the donor had HLA-A*0206, the recipient had HLA-A*0201, while another HLA-A allele of the donor and recipient was identical. This mismatch was compared with the HLA-A allele match. Mismatch combinations which had nine or fewer pairs were combined together as "other mismatch." The model was constructed with mismatch combinations, mismatch status in other loci (match, 1 allele mismatched and 2 alleles mismatched as ordinal variable) and potential confounders. Confounders considered were sex (donor-recipient pair), patient age (linear), donor age (linear), transplant year, type of disease, risk of leukemia relapse (standard, high and diseases other than leukemia), GVHD prophylaxis (cyclosporine (CSP) vs. tacrolimus (FK)), anti-thymocyte globulin (ATG) (ATG vs. no ATG) and preconditioning (total body irradiation (TBI) vs. non-TBI). These

confounders were used in all analyses to maintain the comparability of results.

The impact of position and type of amino acid substitutions in HLA molecules was evaluated in pairs with HLA one-allele mismatched in HLA-Cw and -DPB1 separately. The amino acid positions we analyzed were all positions at which an amino acid was substituted in the respective locus. We analyzed the impact of each amino acid substitution on each position separately. Multivariable models were constructed to include the position and type of amino acid substitution, mismatch status in other loci (match, 1 allele mismatched and 2 alleles mismatched as ordinal variable) and the confounders described above. We applied a P value of less than 0.05 as statistically significant. All statistical tests were two-sided. All analyses were performed using STATA version 10.0 (Stata Corp., College Station, TX) and R version 2.5.1 (The R Foundation for Statistical Computing).

Validation of Statistical Analysis

Statistical analyses were validated using the bootstrap resampling method [14]. Briefly, we estimated the measure of association with re-sampled data drawn repeatedly from the original data. Although around 100 to 200 bootstrapped samplings are generally sufficient [15], we used 1000 bootstrap samples for all analysis validations. Further, we judged the results of analysis as statistically significant only when the results of both base analysis and analysis validation using bootstrap resampling were significant; cases in which the result of base analysis was significant but that of analysis validation using bootstrap resampling was not are indicated by an asterisk next to the P value of the base

analysis.

Results

Impact of HLA allele mismatches in locus level on relapse

The number of mismatched alleles of HLA-Cw (one allele mismatched: Hazard ratio (HR)=0.68, 95% confidence interval (CI): 0.58-0.80; two alleles mismatched: HR=0.43, CI: 0.24-0.75) and HLA-DPB1 (one allele mismatched: HR=0.80, CI: 0.70-0.92; two alleles mismatched: HR=0.62, CI: 0.51-0.75) was strongly associated with a decreased risk of relapse. In contrast, no associations were seen for HLA-A (one allele mismatched: HR=1.00, CI: 0.82-1.22; two alleles mismatched: HR=0.79, 95% CI: 0.28-2.28), HLA-B (one allele mismatched: HR=1.06, 95% CI: 0.79-1.41; two alleles mismatched: not applicable), HLA-DRB1 (one allele mismatched: HR=0.93, 95% CI: 0.74-1.18; two alleles mismatched: HR=1.18, 95% CI: 0.53-2.63) or HLA-DQB1 (one allele mismatched: HR=1.12, 95% CI: 0.90-1.40; two alleles mismatched: HR=0.73, 95% CI: 0.35-1.52) (Figure 1 and Table 2).

Impact of HLA Mismatch Combinations on Relapse

Four HLA mismatch combinations in HLA-Cw and six in HLA-DPB1 were significantly associated with a decreased risk of relapse (Table 3 and supplementary Table 3). In contrast, no significant HLA mismatch combinations were seen for HLA-A, -B, -DRB1 or –DQB1 (data not shown). The 10 associated HLA mismatch combinations were Cw*0102-Cw*1402 (HR not estimated due to no event), Cw*0801-Cw*0102 (HR not estimated), Cw*1402-Cw*0304 (HR not estimated), Cw*1502-Cw*1402 (HR=0.28, 95% CI: 0.09-0.88), DPB1*0402-DPB1*0201

(HR=0.32, 95% CI:0.12-0.87), DPB1*0501-DPB1*0201 (HR=0.67, 95% CI:0.50-0.91), DPB1*0501-DPB1*0401 (HR=0.36, 95% CI:0.13-0.98), DPB1*0501-DPB1*0402 (HR=0.55, 95% CI:0.33-0.93), DPB1*0901-DPB1*0201 (HR=0.37, 95% CI:0.14-0.96), and DPB1*1301-DPB1*0201 (HR not estimated) (Table 3 and supplementary Table 3). All 10 HLA mismatch combinations were also significant on validation analysis using the bootstrap resampling method. We speculated that these mismatch combinations would mainly decrease the risk of relapse due to GVL, so we tentatively call them GVL mismatch combinations.

Evaluation of Clinical Importance of GVL Mismatch Combinations

We evaluated the clinical importance of GVL mismatch combinations in HLA-Cw and –DPB1. All analyses in this section were conducted in matched pairs other than the evaluated locus. In HLA-C mismatch, the small number of patients with GVL mismatch combination (n=13) in matched pairs at the allele level for HLA-A, -B, -DRB1, -DQB1 and -DPB1 prevented comprehensive analysis. We evaluated the GVL mismatch combinations of HLA-DPB1 in matched pairs for HLA-A, -B, -Cw, -DRB1 and -DQB1. Pairs with HLA-DPB1 mismatch were divided into two groups, those with a GVL mismatch combination and those with mismatch combinations other than GVL mismatch combinations. These were then compared with 12/12 matched pairs for association with severe acute GVHD, relapse and overall survival (Table 4). The curve of the cumulative incidence of OS is shown in Figure 2. Multivariable analysis revealed that although OS was closely similar between 12/12 matched pairs and pairs with

mismatch combinations other than GVL mismatch combinations, it was significantly improved in pairs with a GVL mismatch combination (Table 4). In terms of mortality due to relapse according to HLA-DPB1 matching status and whether the mismatch combinations were GVL mismatch combinations, the HLA-DPB1 matched group, HLA-DPB1 1 allele mismatched group and GVL mismatch combination group showed an expected decreased mortality due to relapse (20.0%, 15.3% and 10.5%, respectively). Further, mortality due to relapse in the GVL mismatch combination group was significantly lower than that in the HLA-DPB1 1 allele mismatched group (p=0.049). We conducted the same analyses with stratification by leukemia type (ALL, AML or CML) and found that the myeloid malignancies (AML and CML) had the same tendency (Table 4). In particular, in CML, GVL mismatch combinations in HLA-DPB1 significantly reduced the risk of relapse (HR=0.14, 95% CI:0.03-0.55) and significantly improved OS relapse (HR=0.50, 95% CI:0.25-0.98).

Impact of Position and Type of Amino Acid Substitutions of HLA Molecules on Relapse

We surveyed all substituted positions in HLA-Cw and -DPB1 and found 159 specific amino acid substitutions at 55 positions in HLA-Cw and 55 specific amino acid substitutions at 19 positions in HLA-DPB1 (Supplementary Tables 1 and 2). Analysis revealed three specific amino acid substitutions responsible for a decreased risk of relapse in HLA-C, namely Ser9C-Tyr9C (HR=0.53, 95% CI:0.30-0.92), Phe99C-Tyr99C (HR=0.52, 95% CI:0.30-0.91), and Arg156C-Leu156C (HR=0.59,

95% CI:0.37-0.92). In contrast, no decrease in the risk of relapse was seen for substitutions in HLA-DPB1 (Table 5). However, Tyr9C-Ser9C and Tyr99C-Phe99C were strongly linked (see Discussion). These specific amino acid substitutions were all significant on validation analysis using the bootstrap resampling method.

Discussion

Improving outcomes in allogeneic HSCT for hematological malignancy by separating GVL from GVHD is considered a key clinical challenge. Here, our analysis demonstrated a significant association between several HLA mismatch combinations and specific amino acid substitutions in HLA molecule between donor and recipient with a decreased risk of relapse, some of which did not significantly increase the occurrence of severe acute GVHD. These findings suggest that GVL might be separated from severe acute GVHD by selection of suitable HLA mismatch combinations.

We recently reported 16 significant high-risk HLA allele mismatch combinations for severe acute GVHD in 6 HLA loci, a number of which were highly associated with the occurrence of severe acute GVHD and overall survival[8]. Of note, a group of pairs with mismatches other than severe acute GVHD high-risk mismatches showed an almost equal occurrence of severe acute GVHD and overall survival to 12/12 matched pairs. In the present study, we elucidated a total of 10 mismatch combinations which were significantly associated with a decreased risk of relapse, which we termed GVL mismatch combinations. Of course, it is possible that some mismatch combinations not classified as GVL mismatch combinations might actually induce strong GVL. Misclassification might have occurred as a result of insufficient statistical power due to the relatively small number of subjects in the subcategories. Among these, two of four mismatch combinations in HLA-Cw were identical to severe acute GVHD high-risk combinations; a third had a marginal effect on the occurrence of severe acute GVHD, while the remaining fourth combination was different from acute GVHD high-risk

mismatch combinations. In contrast, all six mismatch combinations in HLA-DPB1 were different from acute GVHD high-risk mismatch combinations (Table 3). As expected, HLA-A, -B, -Cw, -DRB1 and DQB1 matched pairs with GVL mismatch combinations of HLA-DPB1 revealed significantly improved overall survival compared with 12/12 matched pairs (Table 4 and Figure 2), indicating that the beneficial antitumor effect of GVL mismatch combinations in HLA-DPB1 would not be offset by the effect of severe acute GVHD. We speculate that conformation change of HLA molecules in each mismatch combination control the intensity of the acute GVHD and GVL effect, as described below and in our previous manuscript [8]; namely, conformation change of HLA molecules in GVL mismatch combination in HLA-DPB1 induces strong GVL with mild or no acute GVHD. These findings suggest that HLA mismatch selection according to these results might improve HSCT outcomes over those obtained with a complete match. The same tendency was seen for AML and CML, whereas the effect of GVL mismatch combination in the HLA-DPB1 allele in ALL patients would be weaker than in the other leukemia types (Table 4). Comprehensive analyses for ML and MM could ot be done owing to the small number in each group. Thus, the effects of GVL mismatch combination vary according to disease type and may also change according to other factors, including particular cytogenetic abnormalities.

Recent research has shown that HLA-Cw and -DPB1 mismatch at the allele level is strongly associated with a decreased risk of relapse [16,17]. These findings were confirmed in the present large cohort. In addition, the present study also clarified that mismatching of two alleles in either the HLA-Cw or -DPB1 locus had a stronger association with

decreased risk than respective mismatching of one allele. Moreover, no association whatsoever was seen for HLA-A, -B, -DRB1 or -DQB1 (Figure 1 and Table 2). Furthermore, all 10 GVL mismatch combinations were elucidated from mismatch combinations of HLA-Cw and HLA-DPB1 (Table 3 and supplementary Table 3), although we also analyzed HLA-A, -B, -DRB1 and -DQB1. These findings indicate that GVL after allogeneic HSCT is mainly induced by HLA-Cw and -DPB1, not HLA-A, -B, -DRB1 or -DQB1, although the role of each HLA locus might vary with the type of disease [17]. Any of three possible explanations might explain this. First, the relative expression of HLA-Cw and -DPB1 on malignant cells may be higher than that on normal hematopoietic cells; second, HLA-Cw and -DPB1 may be preferentially expressed on malignant stem cells; and third, surface expression of a few key molecules, such as major histocompatibility complex (MHC) molecules, adhesion molecule and costimulatory molecule, on malignant cells may determine the role of each HLA locus on GVL [18-20] – in other words, some molecules might stimulate GVL of HLA-Cw or -DPB1, or other molecules might block GVL of other than HLA-Cw and -DPB1. Further investigation of this question is warranted.

In this study, three specific amino acid substitutions responsible for GVL at positions 9, 99 and 156 were identified in HLA-Cw, of which only two, Ser9C-Tyr9C and Phe99C-Tyr99C, were strongly linked in our sample. We were therefore unable to determine which substitutions are the main contributors to the effect of interest (Table 5). These amino acid positions, 9, 99 and 156, were identical to those we elucidated in our previous study as responsible for severe acute GVHD [8]. These findings suggest that

these three amino acid positions are important determinants of alloreactivity. Although position 156 of the HLA molecule has been shown to modify T-cell alloreactivity in vitro in HLA-A2,[21-23] HLA-B35[24] and HLA-B44[25], to our knowledge our present study is the first to identify positions 9 and 99. On the other hand, substituted amino acids were not necessarily identical. In Ser9C-Tyr9C and Phe99C-Tyr99C substitutions, for example, the substituted amino acid position was identical with that responsible for severe acute GVHD, whereas the substituted amino acids were inverse between donor and recipient, even though both substituted position and amino acids were identical in the Arg156C-Leu156C substitution. These findings suggest that Ser9C-Tyr9C and Phe99C-Tyr99C might play an important role in separating GVL from acute GVHD in HLA-Cw mismatch, although the mechanism requires further molecular study clarification.

With regard to specific amino acid substitutions of HLA-DPB1, we found no significant association among these with a decreased risk of relapse. Shaw et al. reported that mismatches at position 57 and 65 in the HLA-DPB1 molecule were associated with transplant complications, but not with GVHD or relapse rate [26], which is consistent with our present data. We speculate that, compared to MHC class I, the conformation diversity of MHC class II and peptide complex hampers the identification of strict rules between specific amino acid substitutions in MHC class II molecules and the occurrence of alloreaction such as GVHD and GVL. In HLA class I, binding peptides are held by their ends, whereas peptides bind to HLA class II by attachment in the middle, allowing them to be much longer and more variable in length [27].

Given that this analysis was conducted using a Japanese cohort transplanted through the Japan Marrow Donor Program, the applicability of our data to other ethnic groups warrants discussion. We speculate that the effect of alloreaction is a reflection and summation of HLA allele mismatch combinations. Discrepancies in the effect of HLA locus on alloreactions between ethnically-diverse transplantation might be explained by the proportions of each HLA mismatch combination in each HLA locus. In HLA-DPB1, on the other hand, the allele variations between Caucasian and Japanese populations are relatively close, and hence findings in HLA-DPB1 might also be useful for Caucasian populations. Regarding HLA-Cw and killer immunoglobulin-like receptor (KIR) incompatibility, we previously reported adverse effects in unrelated T cell-replete HSCT through the JMDP [17], although Ruggieri et al. demonstrated that beneficial effects were shown in T-cell depleted haploidentical transplant [28]. We speculated that in vivo and/or in vitro T cell depletion could account for this discrepancy [29]. Therefore, results for mismatch combinations in HLA-Cw obtained in other populations treated in other settings may differ from our present results. Nevertheless, clarification of these questions would require the same study in other ethnic populations.

Given the general acceptance that GVL is more closely correlated with chronic GVHD than acute GVHD [3], separating GVL from chronic GVHD may be more difficult than separating it from acute GVHD. On this basis, our results suggest that GVL could be separated from acute GVHD in HSCT from a specific HLA partially mismatched donor. Clarification of whether GVL can also be separated from chronic GVHD requires further study.