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Negative Impact of Unidirectional Host-versus-Graft Killer Cell Immunoglobulin–like Receptor Ligand Mismatch on Transplantation Outcomes after Unmanipulated Haploidentical Peripheral Blood Stem Cell Transplantation for Acute Myeloid Leukemia

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This study explored the influence of mismatched inhibitory killer cell immunoglobulin–like receptor (KIR) ligands on the outcome of haploidentical transplantation using T cell–replete, granulocyte colony–stimulating factor–mobilized peripheral blood stem cells in adult patients with acute myeloid leukemia (AML). Three groups were examined: unidirectional graft-versus-host KIR ligand mismatched (GVH-KIR-MM; n = 33), bidirectional KIR ligand matched (KIR-M; n = 41), and unidirectional host-versus-graft KIR ligand mismatched (HVG-KIR-MM; n = 26). All recipients were treated with the same conditioning regimen (800 cGy total body irradiation, fludarabine, busulfan, and antithymocyte globulin). After a median follow-up of 26 months, the 2-year cumulative incidence of relapse was significantly higher in HVG-KIR-MM (40.3% ± 10.3%) versus others (18.9% ± 4.8%, $P = .044$). In the standard-risk group, the 2-year disease-free survival (DFS) was significantly lower in HVG-KIR-MM (51.8% ± 11.2%) compared with GVH-KIR-MM (88% ± 8.1%, $P = .025$). Multivariate analysis showed that HVG-KIR-MM was significantly associated with higher relapse (hazard ratio [HR], 10.7; $P = .002$) and lower DFS (HR, 3.4; $P = .012$). Subgroup analysis revealed increased DFS with higher doses of $CD3^+CD8^+$ and $CD3^-CD56^+$ grafts in GVH-KIR-MM (90.9% ± 8.7%, $P = .006$); there was no such effect in the other groups. Although our conclusions are limited by the absence of donor KIR genotype data, our study suggests unidirectional KIR ligand incompatibility in the host-versus-graft vector has a detrimental effect on T cell–replete haploidentical transplantation outcomes in adult patients with AML.

INTRODUCTION

Major histocompatibility complex–restricted donor immune cells play a curative role in allogeneic hematopoietic stem cell transplantation (HSCT) for hematologic malignancies by recognizing differences in a broad array of polymorphic major and minor histocompatibility antigens between donor and recipient [1,2]. Recent progress in the understanding of natural killer (NK) cell receptor biology suggests the contribution of NK cell alloreactivity to this graft-versus-leukemia effect [3-5]. NK cells are regulated by a net balance of activating and inhibitory signals that finely tune potent effector functions, such as cytolytic activity [6,7]. Human NK cells express inhibitory killer cell immunoglobulin–like receptors (KIRs) that recognize specific groups of HLA class I alleles as allotypic determinants ("KIR ligands") and are thought to engage the potential target cell through

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activating receptors [8-10]. KIR and KIR ligand induction of NK cell alloreactivity in haploidentical (haplo) HSCT setting was first demonstrated by the Perugia group based on donor and recipient KIR ligands (ligand-ligand model) fulfilling the criterion of "missing self" [11,12]. Because KIR genes are not present in all individuals [13] and KIR gene expression may vary [14], Leung et al. introduced the receptor-ligand model defined by donor KIR genotype and phenotype assessment with recipient HLA genotype to avoid errors from interindividual variability [14,15]. However, subsequent clinical studies showed conflicting results, perhaps due to different transplantation strategies [16-19]. The significance of ethnicity also remains an open issue [20,21]. The aim of this retrospective study was to assess the influence of KIR ligand status on the outcome of 103 consecutive patients with acute myeloid leukemia (AML) who underwent haplo-HSCT using unmanipulated peripheral blood stem cells (PBSCs) with the same conditioning regimen and graft-versus-host disease (GVHD) prophylaxis.

PATIENTS AND METHODS

Subjects and Clinical Data

This study was conducted according to the Declaration of Helsinki and included 103 consecutive adult recipients with AML who underwent haplo-HSCT from a family donor at Catholic Blood and Marrow Transplantation Center between September 2008 and May 2014. Recipients whose donors were mismatched for at least 2 or more HLA-A, -B, -C, and/or -DR loci were evaluated to analyze the influence of KIR ligand compatibility status on the incidence of relapse after transplantation. All recipients received a conditioning regimen composed of a fractionated 800 cGy total body irradiation, fludarabine (30 mg/m^2/day for 5 days), i.v. busulfan (3.2 mg/kg/day for 2 days), rabbit antithymocyte globulin (ATG; 1.25 mg/kg/day for 4 days), and GVHD prophylaxis with tacrolimus plus short-course methotrexate. Unmanipulated granulocyte colony–stimulating factor (G-CSF)–mobilized PBSCs were used for graft source. Other transplantation procedure and supportive care were performed as previously described [22].

Because donor KIR genotypes were not available for this study, KIR ligand status was determined by assuming that an individual possesses a full complement of inhibitory receptors for KIR [23,24]. For the purpose of outcome analysis, KIR-driven alloreactivity in the graft-versus-host (GVH) direction was predicted when the donor expresses (but the recipient lacks) a KIR ligand for the corresponding inhibitory KIR and vice versa for that in the host-versus-graft (HVG) direction. High-resolution typing of recipient/donor HLA-B and -C alleles was used to assign recipients to the following inhibitory KIR ligand groups as described by Ruggeri et al. [12]: HLA-C group 1 alleles (C1) recognized by KIR2DL2 and 2DL3; HLA-C group 2 alleles (C2) specific for KIR2DL1; and HLA-Bw4-positive alleles (Bw4) recognized by KIR3DL1. We also considered HLA-A3/-A11 alleles known as putative KIR3DL2 ligands [25].

Statistical Analysis

Time to event was assessed from the infusion day. Events for disease-free survival (DFS) were relapse or death from any cause, whereas death from any cause was a relevant event for overall survival (OS). *Transplantation-related mortality* (TRM) was defined as death from any cause during continuous remission. Categorical and continuous variables were compared by the chi-square or Fisher's exact test and 1-way analysis of variance, respectively. Probabilities of OS and DFS were calculated using the Kaplan-Meier method and compared by the log-rank test. Probabilities of neutrophil and platelet recovery, acute and chronic GVHD, relapse, and TRM were estimated using the cumulative incidence method and compared by the Gray test. GVHD was diagnosed and graded according to clinical consensus criteria [26,27].

Adjusted outcome probabilities were estimated using the Cox proportional hazards regression (OS, DFS) and Fine and Gray (relapse and TRM) models with consideration of the variables in the final multivariate models. Variables with P value $< .10$ in the univariate Cox analyses were considered for inclusions in the multivariate models constructed using a step-wise model selection; variables were retained in the final model if $P < .05$. KIR ligand compatibility was retained in all steps of model building. Other variables included recipient age, donor age, sex, sex match, cytogenetic risk [28] disease status at HSCT, donor-recipient relation, HLA disparity, and graft cell doses (dichotomized as "lower" versus "higher" according to median values). All statistical analyses were conducted using R.3.1.1 statistical software and SPSS (SPSS Inc., Chicago, IL).

RESULTS

Frequency of KIR-epitope Homozygosity

All recipients and donors were of Korean ethnicity. The subjects (recipients/donors, n = 206) included 23 HLA-A, 43 HLA-B, 25 HLA-C, and 33 HLA-DR alleles. All cases analyzed in this study were mismatched for at least 2 different alleles on HLA-A, -B, -C, and/or -DR loci: 74 (72%) donor/recipient pairs were mismatched for HLA-A, -B, and -C alleles (also HLA-DR mismatched in 68 of 74 pairs); 25 (23%) pairs were mismatched for HLA-A/-B, HLA-A/-C, or HLA-B/-C (also HLA-DR mismatched in 22 pairs); 5 (5%) pairs were mismatched for 1 of HLA-A, -B, or -C loci and HLA-DR. In our cohort, the donor and recipient HLA-C KIR ligand types were C1C1 in 150 (73%), C1C2 in 37 (25%), and C2C2 in 4 (2%) subjects. Similar proportions were observed in the recipients (C1C1, n = 76 [74%]; C1C2, n = 25 [24%]; C2C2, n = 2 [2%]) and donors (C1C1, n = 74 [72%]; C1C2, n = 27 [26%]; C2C2, n = 2 [2%]). Bw4 homozygosity on HLA-B was seen in 6 (6%) donors and 11 (11%) recipients; heterozygosity was observed in 68 (66%) donors and 61 (59%) recipients. All but 2 cases (1 donor/recipient pair, HLA-A*2420) of HLA-A Bw4 epitope were HLA-A*2402. Lastly, 24 (23%) donors and 23 (22%) recipients had HLA-A3 or -A11.

Donor/Recipient KIR Ligand Status

Donor KIR genotypes were not available for this study; therefore, KIR ligand compatibility was predicted by assuming that an individual possesses a full complement of inhibitory receptors for KIR27, 28 and, for the purpose of outcome analysis, NK alloreactivity in the GVH direction was predicted when the donor expresses (but the recipient lacks) a KIR ligand (C1, C2, Bw4, or A3/A11) for the corresponding inhibitory KIR, and vice versa for NK alloreactivity in the HVG direction. A combination of donor/recipient HLA-A, -B, and -C genotype results showed 36 (35%) donor/recipient pairs with KIR ligand incompatibility (HLA-A, -B, and/or -C) in the GVH direction and 29 (28%) pairs in the HVG direction. Three of these pairs showed bidirectional KIR ligand incompatibility. The remaining 41 donor/recipient pairs had compatible KIR ligands in both directions. We then divided the recipients according to the direction of KIR ligand mismatch as follows: recipients with KIR ligand mismatch for donor KIR ligands in the GVH direction only (GVH-KIR-MM), n = 33 (33%); recipients with KIR ligand matched with donor KIR ligands in both directions (KIR-M), n = 41(41%); and recipients with KIR ligand mismatch for donor KIR ligands in the HVG direction only (HVG-KIR-MM), n = 26 (26%). The clinical characteristics of these groups demonstrated that the number of recipients with advanced-risk disease status at transplantation were lower in the HVG-KIR-MM group (15% versus 42% in GVH-KIR-MM and 44% in KIR-M; $P = .039$), whereas the number of recipients with mismatched HLA alleles ≤ 2 in HVG direction was higher in the KIR-M group (27% versus 6% in GVH-KIR-MM and 8% in HVG-KIR-MM; $P = .022$). Other baseline clinical characteristics between these 3 groups were similar (Table 1). Because of the small number (n = 3), recipients with bidirectional KIR ligand incompatibility were excluded from further analyses.

Engraftment and GVHD

All recipients achieved neutrophil recovery (median, 11 days; range, 9 to 42) and all recipients showed platelet