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Expression of Activating KIR2DS2 and KIR2DS4 Genes after Hematopoietic Cell Transplantation: Relevance to Cytomegalovirus Infection

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The important role of activating killer immunoglobulin-like receptors (KIRs) in protecting against cytomegalovirus (CMV) reactivation has been described previously in patients undergoing hematopoietic cell transplantation (HCT). More specifically, the presence of multiple activating KIRs and the presence of at least KIR2DS2 and KIR2DS4 in the donor genotype identified a group of HCT patients at low risk for CMV reactivation. However, CMV infection still occurs in patients with the KIR protective genotype, and the question has been raised as to whether this is related to the lack of KIR expression. In this report, expression of the KIR2DS2 and KIR2DS4 genes, as measured by mRNA-based quantitative polymerase chain reaction in both the donor cells and the HCT recipient cells, was studied relative to CMV reactivation. In the control samples from healthy donors, the median range for KIR2DS2 and KIR2DS4 expression was low, with 35% of donors considered null-expressers. Interestingly, KIR2DS2 and KIR2DS4 expression was elevated after HCT compared with donor expression before HCT, and was significantly elevated in CMV viremic compared with CMV nonviremic HCT recipients. The CMV seropositivity of donors was not associated with activating KIR expression, and donor null expression in those with the KIR2DS2 or KIR2DS4 genotype was not predictive for CMV reactivation in the recipient. After controlling for other transplant factors, including donor type (sibling or unrelated), transplant source (bone marrow or peripheral blood stem cells), and acute GVHD grade, regression analysis of elevated KIR gene expression found an association for both KIR2DS2 and KIR2DS4, with a 7-fold increase in risk for CMV reactivation. We speculate that the elevated activating KIR expression in CMV-viremic HCT recipients is either coincidental with factors that activate CMV or is initiated by CMV or cellular processes responsive to such CMV infection reactivation.

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INTRODUCTION

Previous reports have emphasized the importance of natural killer (NK) cells and their killer cell immunoglobulin-like receptors (KIRs) in controlling cytomegalovirus (CMV) reactivation after hematopoietic cell transplantation (HCT). More specifically, the use of donors with more than one activating KIR (aKIR) gene were associated with a 65% reduction in CMV reactivation [1,2], and the same effect has been reported in kidney transplantation [3]. In the HCT setting, the number of aKIR genes in the donors, but not in the recipients, has been associated with protection against CMV reactivation, with the greatest protective effect when the donor genotype contained >5 aKIR genes or at least a combination of KIR2DS2 and KIR2DS4 [4].

The aKIR genotype profile is not necessarily correlated with concurrent expression, however. Whether aKIR expression predicts protection from CMV and, conversely, whether the nonexpression of these genes explains the failure of a KIR2DS2/KIR2DS4 genotype to protect from CMV infection is unclear. In the present work, we evaluated the expression of the donor

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aKIR genes KIR2DS2 and KIR2DS4 in recipients of allogeneic HCT. We analyzed the effect of the HCT regimen as well as cell reconstitution with aKIR gene expression to address the foregoing questions and to test whether expression levels are modulated by post-HCT events, such as CMV reactivation or graft-versus-host disease (GVHD). This report focuses on activating KIR2DS2 and KIR2DS4 gene expression, because this dual aKIR genotype appears to be protective, and examines the expression relative to CMV infection in recipients of allogeneic HCT.

Although KIR expression can be detected by flow cytometry, the available antibodies do not differentiate the activating KIR2DS2 and its linked gene, the inhibitory KIR2DL2. In the present study, we used an mRNA-based quantitative polymerase chain reaction (Q-PCR) method for this purpose. Previous reports have described specific primers for detecting KIR alleles for genotyping, which also have been used to detect mRNA and cDNA by various methods, including real-time Q-PCR [5-7]. These methods have limitations, however, and not all published primers can amplify KIR cDNA using the Q-PCR method, especially if the amplified sequence is large (i.e., >200-300 bp). In the present work, we developed a method for quantifying KIR2DS2 and KIR2DS4 gene expression using previously cryopreserved peripheral blood mononuclear cells (PBMCs) and for establishing a baseline of aKIR gene expression in donor samples with a corresponding genotype before HCT. Activating KIR expression was followed longitudinally at various times posttransplantation, and the relationship of aKIR expression to CMV reactivation and to GVHD was evaluated. We found that CMV infection, but not GVHD, was associated with increased aKIR gene expression.

MATERIALS AND METHODS

Study Patients

In a previous study, 211 consecutive allogeneic HCT recipients who underwent transplantation between 2001 and 2006 were followed for CMV reactivation for at least 1 year posttransplantation [4]. From that study, cryopreserved PBMC samples from 134 HCT recipients with the *KIR2DS2* or *KIR2DS4* genotype, or both, were available for further analysis at days 40, 90, 120, 150, and 180 post-HCT. In addition, 81 donor samples were also available, of which 55 were from donor–recipient pairs. Based on study eligibility, all HCT recipients were at risk for CMV reactivation as determined by CMV antibody seropositivity in the donor, the recipient, or both. All subjects (both donors and recipients) provided signed informed consent for research approved by the City of Hope's Institutional Review Board, and the use of the leftover specimens for this study is covered under an Institutional Review Board protocol. Table 1 summarizes general demographic and clinical data for the 134 study participants in terms of median age, transplant type, disease diagnosis, myeloablative versus nonmyeloablative conditioning, and GVHD prophylaxis.

CMV Reactivation

CMV reactivation was monitored by DNA Q-PCR on plasma collected twice weekly up to 100 days post-HCT. CMV surveillance was continued at 1- to 2-week intervals in "high-risk" patients based on clinical management guidelines at City of Hope. High-risk patients included those with persistent lymphopenia, with grade II-IV GVHD, or requiring continued immunosuppression for anti-GVHD therapy. Q-PCR was performed essentially as described previously using CMV-gB DNA as an amplification product [8], with a lower limit of detection of 200 genome copies (gc)/mL of plasma. In addition to Q-PCR testing on plasma, all patients had whole blood cultured for CMV infection on the same blood specimen using a shell vial culture method as described previously [9]. In this study, CMV infection was defined as evidence of CMV in blood culture shell vial, plasma Q-PCR, or

Table 1. Demographic Data (n = 134)

Recipient age at transplantation, years, median (range)	42.1 (18.8-63.6)
Donor age at transplantation, years, median (range)	42.1 (18.0-71.4)
Donor type, n (%)	
Sibling donor	87 (65%)
Unrelated donor	47 (35%)
Stem cell source, n (%)	
BM	24 (18%)
PBSCs	110 (82%)
Diagnosis, n (%)	
Lymphoid	51 (38%)
Myeloid	77 (57%)
Other	6 (5%)
Disease status at transplantation, n (%)	
First CR/CP	57 (43%)
Later CR/CP	16 (12%)
Relapse	22 (16%)
Induction failure	22 (16%)
MDS, AA, MM, or MPD	17 (13%)
Conditioning, n (%)	
Fludarabine/melphalan	45 (34%)
Myeloablative	88 (66%)
CMV serology, n (%)	
Missing	1
D^-/R^+	33 (25%)
D^+/R^-	14 (10%)
D^+/R^+	86 (65%)
aGVHD grade, n (%)	
0-I	53 (40%)
II-IV	81 (60%)
Chronic GVHD grade, n (%)	
None	21 (16%)
Limited	15 (11%)
Extensive	98 (73%)

AA indicates aplastic anemia; CP, chronic phase; CR, complete remission; MDS, myelodysplastic syndrome; MM; multiple myeloma; MPD, myeloproliferative disorder.