Cytomegalovirus Reactivation After Matched Sibling Donor Reduced-Intensity Conditioning Allogeneic Hematopoietic Stem Cell Transplant Correlates With Donor Killer Immunoglobulin-like Receptor Genotype

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Abstract

Objectives: **Cytomegalovirus reactivation is common after reduced-intensity conditioning allogeneic hematopoietic stem cell transplant. Natural killer and T cells mediate immunity against viruses including *cytomegalovirus*. The alloreactivity of Natural killer cells and some T-cell subsets is mediated through the interaction of their killer immunoglobulin-like receptors with target cell ligands. This study sought to assess whether donor inhibitory or activating killer immunoglobulin-like receptor genotypes may influence post-transplant *cytomegalovirus* reactivation in transplant recipients.**

Materials and Methods: **We analyzed 64 patients who underwent T-cell replete, matched sibling donor reduced-intensity conditioning allogeneic hematopoietic stem cell transplantation at our institution. Transplant recipients were categorized according to their HLA inhibitory killer immunoglobulin-like receptor ligand groups. Donor killer immunoglobulin-like receptor genotypes were determined and then were assessed for correlations with *cytomegalovirus* reactivation in transplant recipients.**

Results: **No differences in *cytomegalovirus* reactivation were observed when comparing those with or without missing inhibitory killer immunoglobulin-like receptor ligands. When considering the number of donor activating killer immunoglobulin-like receptor genes, those with 5 or 6 had less *cytomegalovirus* reactivation than those with 1 to 4 (19% vs 48%; *P* = .029). The difference could not be attributed to baseline patient or transplant characteristics. No specific activating killer immunoglobulin-like receptor genotype was found to be associated with *cytomegalovirus* reactivation.**

Conclusions: **These observations indicate that assessment of donor killer immunoglobulin-like receptor genotype may have important implications for predicting *cytomegalovirus* reactivation after T-cell replete, matched sibling donor reduced-intensity conditioning allogeneic hematopoietic stem cell transplant.**

Key words: Cytomegalovirus, *Killer immunoglobulin-like receptors, Reduced-intensity conditioning, Allogeneic hematopoietic stem cell transplant, Matched sibling donor*

Reduced-intensity conditioning allogeneic hematopoietic stem cell transplant is effective for many patients who are at high risk for transplant-related mortality with myeloablative allogeneic hematopoietic stem cell transplant.[1-4] Although reduced-intensity conditioning allogeneic hematopoietic stem cell transplant may avoid many of the organ toxicities associated with myeloablative conditioning, the risk for developing graft-versus-host disease and infection including *cytomegalovirus* remains significant.[5]

Cytomegalovirus is the most-common viral infection after allogeneic hematopoietic stem cell transplant. Natural killer (NK) and T cells provide

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protection against *cytomegalovirus* reactivation.[6, 7] The reactivity of NK cells and some T-cell subsets are regulated by the interaction of killer immunoglobulin-like receptors with target cell HLA-class 1 molecules.[8] Killer immunoglobulin-like receptors exist in either an activating or inhibitory form, both of which share a common 2 or 3 subunit extracellular domain. Inhibitory or activating signals are generated when a ligand engages the respective inhibitory or activating killer immunoglobulin-like receptor of an immune effector cell. Different activation/inhibitory signaling pathways are controlled by interactions through these receptors, and their balance regulates the behavior of the NK cell.[9]

Human leucocyte antigen-Cw is the main ligand for most inhibitory killer immunoglobulin-like receptors. This includes a C1 and C2 group with the designations based on the amino acid residues at positions 77 and 80 in the α1 helix of the HLA-C molecule.[10] The C1 group is characterized by a serine at position 77 and an asparagine at position 80. This group includes HLA-Cw1, -Cw3, -Cw7, and -Cw8, and the respective inhibitory receptors are KIR2DL2 and KIR2DL3. In contrast, the C2 group has an asparagine at position 77 and a lysine at position 80. This group includes HLA-Cw2, -Cw4, -Cw5, and -Cw6, and the corresponding inhibitory receptor is KIR2DL1. Other human inhibitory killer immunoglobulin-like receptors with known ligands include KIR3DL1 that binds to HLA-Bw4 epitopes[11] and KIR3DL2 that binds to HLA-A3 or HLA-A11.[8] The natural ligands for activating killer immunoglobulin-like receptors are poorly defined, and their affinity for binding to HLA molecules is considerably weaker than that for inhibitory killer immunoglobulin-like receptors.[12]

Killer immunoglobulin-like receptor interactions have been suggested to influence outcomes after myeloablative allogeneic hematopoietic stem cell transplant.[13, 14] We have reported that killer immunoglobulin-like receptor/ligand matching also may be important for reduced-intensity conditioning allogeneic hematopoietic stem cell transplant where we observed it to influence achievement of complete donor T-cell chimerism and development of graft rejection.[15]

The donor activating killer immunoglobulin-like receptor genotype has been implicated as a contributing factor for *cytomegalovirus* reactivation after myeloablative allogeneic hematopoietic stem cell transplant.[16-18] This report suggests that donor killer immunoglobulin-like receptor genotypes also may influence reactivation of *cytomegalovirus* after T-cell replete, matched sibling donor reduced-intensity conditioning allogeneic hematopoietic stem cell transplant.

Materials and Methods

Patient characteristics

We analyzed 64 consecutive patients who underwent T-cell replete, matched sibling donor reduced-intensity conditioning allogeneic hematopoietic stem cell transplant at our institution from January 16, 2000 through April 24, 2007. All patients and donors were treated on reduced-intensity conditioning allogeneic hematopoietic stem cell transplant protocols that were reviewed and approved by the Cleveland Clinic's Institutional Review Board, and the protocols conformed with the ethical guidelines of the 1975 Helsinki Declaration. Signed informed consent was obtained from all patients before the transplant.

Treatment

All patients received fludarabine 30 mg/m^2 once daily on days -5, -4 and -3 and then low-dose total body irradiation as follows: 200 cGy on day -1 (n=36), or 200 cGy on days -1 and 0 (total dose 400 cGy; n=28). Donors received G-CSF 10 mcg/kg subcutaneously daily for peripheral blood stem cell mobilization. Leukapheresis began on the fifth day of G-CSF administration and continued for 1 or 2 days to provide a minimum of 2.0 × 10^6 CD34+ cells/kg for transplant. All transplants were performed using T-cell replete, allogeneic peripheral blood stem cells. Graft-versus-host disease prophylaxis consisted of cyclosporine 100 mg twice daily starting on day -1 and mycophenolate mofetil 500 mg 3 times per day starting on day +1. In the absence of graft-versus-host disease, mycophenolate mofetil was discontinued on day +56, while cyclosporine was tapered beginning on day +100 until discontinuing by day +180. *Cytomegalovirus* monitoring was performed with the Digene hybrid capture *cytomegalovirus* DNA quantitative assay (Digene Corporation, Gaithersburg, MD, USA).

Cytomegalovirus assessment

Cytomegalovirus assessments were done on whole blood using a quantitative *Cytomegalovirus* Digene

Hybrid capture assay (Digene Corporation).[19] *Cytomegalovirus* reactivation was defined as any detection of *cytomegalovirus* DNA in the blood; the lower detection limit for this assay was 600 copies/mL.

Killer immunoglobulin-like receptor genotyping

Human leucocyte antigen typing on donors and recipients was performed as previously described to allow assessment of killer immunoglobulin-like receptor ligands.[15] Donor killer immunoglobulin-like receptor genotypes were determined by PCR-SSOP (One Lambda, Canoga Park, CA, USA) and/or PCR-SSP (Invitrogen, Carlsbad, CA, USA) using commercial kits according to the manufacturer's instructions. This typing provided data for the presence or absence of killer immunoglobulin-like receptor genes and limited information about particular killer immunoglobulin-like receptor alleles or variants.

Patients were categorized according to their HLA inhibitory killer immunoglobulin-like receptor ligand groups by determining whether or not they expressed (1) HLA-A3 or -A11; (2) HLA-Bw4; and (3) HLA-Cw groups (homozygous C1, homozygous C2, or heterozygous C1/C2).[8, 10, 11] All of the donor-recipient pairs had DNA samples available from which killer immunoglobulin-like receptor typing was performed retrospectively to determine the donor inhibitory killer immunoglobulin-like receptor (KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL1, and KIR3DL2) and activating killer immunoglobulin-like receptor (KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, and KIR3DS1) genotypes. For the purposes of this analysis, donors with KIR2DS4 as the only activating gene were classified as killer immunoglobulin-like receptor A/A genotype, whereas those who had more than 1 activating killer immunoglobulin-like receptor gene were classified as the killer immunoglobulin-like receptor B/X genotype.[20, 21]

Statistical analyses

Categoric variables are summarized as frequency counts and percentages and compared between groups using the chi-square test. Continuous variables are summarized as the median and range and compared between groups using either the *t* test or Wilcoxon signed rank test. The Kaplan-Meier method was used to estimate rates of *cytomegalovirus* reactivation after reduced-intensity conditioning allogeneic hematopoietic stem cell transplant, and groups were compared using the log-rank test.

Next, Cox proportional hazards analysis was used to identify multivariable prognostic factors for *cytomegalovirus* reactivation. A stepwise selection procedure was used with a variable entry criterion of $P < .10$ and a variable retention criterion of $P < .05$. The following variables were considered in the analysis, regardless of their significance in univariable analysis: age at transplant, sex, race, diagnosis, prior radiation, total body irradiation dosage used for transplant (200 or 400 cGy), donor-to-recipient sex, grade 2 to 4 acute graft-versus-host disease, missing inhibitory killer immunoglobulin-like receptor ligands (C1, C2, Bw4, A3/11), and number of donor activating killer immunoglobulin-like receptors. Results are summarized as the hazard ratio, 95% confidence interval, and *P* value. All analyses were done using SAS® software. All statistical tests were 2-sided, and $P < .05$ was used to indicate statistical significance.

Results

Patient characteristics

The median age was 55 years (range, 29-67 years) and 34 were men (53%). Most patients were white (n=58), while 6 were African American. Primary diagnoses included 12 acute myeloid leukemia, 12 non-Hodgkin lymphoma, 9 myelodysplastic syndromes, 7 multiple myelomas, 5 chronic lymphocytic leukemias, 4 chronic myeloid leukemias, 4 myelofibrosis, 4 renal cell carcinomas, 3 Hodgkin lymphomas, 1 aplastic anemia, 1 acute lymphoblastic leukemia, 1 congenital anemia, and 1 myeloproliferative disorder. The median time from diagnosis to transplant was 14 months (range, 2 to 174 months).

***Cytomegalovirus* status and reactivation**

Forty-nine patients (77%) were *cytomegalovirus* seropositive or had a *cytomegalovirus* seropositive donor. Twenty-five of them had *cytomegalovirus* reactivation posttransplant (51%). Only 1 patient developed *cytomegalovirus* tissue invasive disease (colitis), and all other cases were *cytomegalovirus* viremia. Fifteen patients (23%) were *cytomegalovirus* seronegative along with their donors. One of them (7%) had *cytomegalovirus* viremia posttransplant.

Killer immunoglobulin-like receptor genotyping

Thirty-three patients (52%) were homozygous for either the C1 or C2 group. The remaining 31 patients who were C1/C2 heterozygotes tended to have less *cytomegalovirus* reactivation posttransplant than the homozygous patients (P = .08). Missing patient inhibitory killer immunoglobulin-like receptor ligands were observed in 23 of the C1 group (36%), 9 of the C2 group (14%), 25 of the HLA-Bw4+ patients (39%), and 43 of the HLA-A3/11+ patients (67%). There were no differences observed for *cytomegalovirus* reactivation between any of these groups when comparing those with or without missing inhibitory killer immunoglobulin-like receptor ligands.

When the number of donor activating killer immunoglobulin-like receptor genes was considered though a difference in *cytomegalovirus* reactivation patterns was appreciated. Patients whose donor killer immunoglobulin-like receptor genotype contained 5 or 6 activating killer immunoglobulin-like receptor genes (n=16) had 3 cases (19%) of *cytomegalovirus* reactivation compared with 23 (48%) of those with only 1 to 4 activating killer immunoglobulin-like receptor genes (n=48) (Figure 1). No specific activating killer immunoglobulin-like receptor was found to be associated with *cytomegalovirus* reactivation. There was no difference in *cytomegalovirus* reactivation when donors with a killer immunoglobulin-like receptor A/A genotype (n=18) were compared with those with killer immunoglobulin-like receptor B/X (n=46) genotype. In addition, there were no differences in *cytomegalovirus* reactivation when comparing those with 1 versus > 1 or 1 to 2 versus 3 to 4 donor activating killer immunoglobulin-like receptors. For those patients who developed *cytomegalovirus* reactivation, the median times to its occurrence was 2.7 months (range, 0.4 to 27.1 months) versus 3.3 months (range, 0.4 to 9.7 months) for the 1 to 4 and the 5 to 6 activating killer immunoglobulin-like receptor gene groups (P = 1.0).

The difference observed in *cytomegalovirus* reactivation between those whose donors had 1 to 4 or 5 to 6 activating killer immunoglobulin-like receptor genes could not be attributed to baseline patient or transplant characteristics (Tables 1 and 2). For the 1 to 4 and the 5 to 6 activating killer immunoglobulin-like receptor gene groups there were 23 cases (48%) and 3 cases (19%) of grade 2 to 4 acute graft-versus-host disease (P = .043). However, only 8 of the cases for the 1 to 4 activating killer immunoglobulin-like receptor gene group developed grade 2 to 4 acute graft-versus-host disease before *cytomegalovirus* reactivation. There were no differences in the incidences of grade 3 to 4 acute graft-versus-host disease, any chronic or extensive chronic graft-versus-host disease between those with

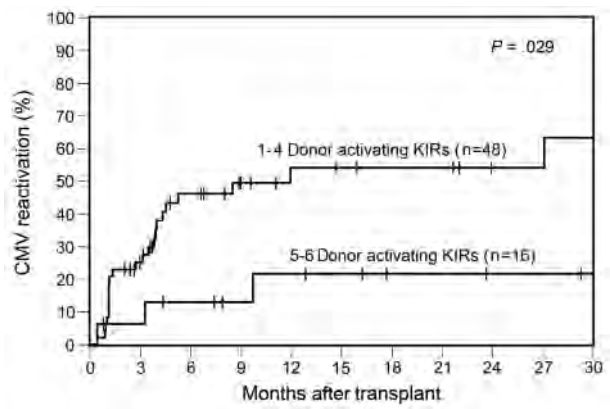

Figure 1. Kaplan-Meier curves for *cytomegalovirus* reactivation after reduced-intensity conditioning allogeneic hematopoietic stem cell transplant based on the number of donor activating killer immunoglobulin-like receptor genes.
Abbreviations: CMV, *cytomegalovirus;* KIR, killer immunoglobulin-like receptor

Table 1. Comparison of patient characteristics based on the number of donor activating killer immunoglobulin-like receptors.

Variable	1 to 4 aKIRs (n=48) n (%)	5 to 6 aKIRs (n=16) n (%)	*P* value
Age at transplant (yrs)			
Median (range)	56 (29-67)	54 (34-62)	.43
Sex			
Male	25 (52)	9 (56)	.77
Female	23 (48)	7 (44)	
Race			
White	43 (90)	15 (94)	.62
African American	5 (10)	1 (6)	
Diagnostic category a			
Myeloid	21 (49)	9 (60)	.46
Lymphoid	22 (51)	6 (40)	
Months from diagnosis to transplant			
Median (range)	16 (2-162)	11 (3-174)	.22
Prior radiation therapy	9 (19)	2 (13)	.57
Number of prior chemotherapy regimens			
0	7 (15)	3 (19)	.85
1	10 (21)	5 (31)	
2	13 (27)	4 (25)	
3	7 (15)	2 (13)	
≥ 4	11 (23)	2 (13)	
Prior transplants	10 (21)	2 (13)	.46
Recipient CMV seropositive	32 (67)	8 (50)	.23
Donor CMV seropositive	28 (58)	10 (63)	.77
Recipient or donor CMV seropositive	38 (79)	11 (69)	.39

a - n excludes renal cell carcinoma, aplastic anemia, and congenital anemia patients
Abbreviations: aKIR, activating killer immunoglobulin-like receptor; CMV, *cytomegalovirus*

Table 2. Comparison of transplant characteristics based on the number of donor activating killer immunoglobulin-like receptors.

Variable	1 to 4 aKIRs (n=48) n (%)	5 to 6 aKIRs (n=16) n (%)	*P* value
Donor to recipient sex			
Female to female	12 (25)	5 (31)	.69
Female to male	12 (25)	3 (19)	
Male to female	11 (23)	2 (13)	
Male to male	13 (27)	6 (37)	
Total nucleated cell dose (× 10^8/kg)			
Median (range)	11.14 (2.06-21.02)	9.03 (4.26-17.79)	.40
CD34+ cell dose (× 10^6/kg)			
Median (range)	5.89 (2.21-12.51)	5.71 (2.04-7.04)	.31
CD3+ cell dose (× 10^8/kg)			
Median (range)	3.95 (1.08-8.18)	4.25 (1.08-8.12)	.48
CD8+ cell dose (× 10^8/kg)			
Median (range)	1.20 (0.17-3.05) [a]	1.16 (0.16-3.19) [b]	.94
Total body irradiation dose			
200 cGy	27 (56)	9 (56)	1.0
400 cGy	21 (44)	7 (44)	

a – n=41; b – n=11
Abbreviations: aKIR, activating killer immunoglobulin-like receptor

1 to 4 or 5 to 6 activating killer immunoglobulin-like receptor genes.

On univariable analysis, there was a trend for the number of donor activating killer immunoglobulin-like receptor genes per 1 unit increase to predict for *cytomegalovirus* reactivation (P = .08; HR 0.79; 95% CI 0.61-1.03). On multivariable analysis, total body irradiation dose 200/400 cGy (HR 2.62; 95% CI 1.05-6.54; P = .039) was a significant predictor of *cytomegalovirus* reactivation, and 5 to 6 versus 1 to 4 donor activating killer immunoglobulin-like receptor genes (HR 0.31; 95% CI 0.09-1.07; P = .06) tended to be also. No other factors were significant on multivariable analysis.

Cytomegalovirus reactivation occurred in 20 patients (56%) who had received 200 cGy total body irradiation and 6 patients (21%) who had 400 cGy total body irradiation (P = .03). As compared with the 400 cGy total body irradiation group, the 200 cGy patients received a higher median CD34+ cell dose (4.93 vs 6.89 × 10^6/kg; P < .001). However, there were no differences between the total body irradiation dose groups for grades 2 to 4 or 3 to 4 acute graft-versus-host disease, chronic graft-versus-host disease, extensive chronic graft-versus-host disease, age, time from diagnosis to transplant, total nucleated cell dose, CD3+ cell dose, time to achieve T-cell complete donor chimerism, sex, race, myeloid versus lymphoid diagnosis, prior radiation therapy, number of prior chemotherapies, recipient or donor *cytomegalovirus* seropositive status, donor to recipient sex relationship, or 1 to 4 versus 5 to 6 donor activating killer immunoglobulin-like receptors.

Discussion

Cytomegalovirus infection is common after both myeloablative[21-23] and reduced-intensity conditioning[5] allogeneic hematopoietic stem cell transplant. Natural killer cells and T cells mediate immunity against viruses including *cytomegalovirus*.[6,7] The alloreactivity of donor-derived NK cells and some T-cell subsets is mediated through the interaction of their killer immunoglobulin-like receptors with recipient HLA-KIR ligands. These interactions have been suggested to influence outcomes after myeloablative[13, 14, 24-27] and reduced-intensity conditioning[15] allogeneic hematopoietic stem cell transplant.

In matched sibling donor allogeneic hematopoietic stem cell transplant, although donors and recipients have identical HLA types, the loci for HLA and killer immunoglobulin-like receptors are on different chromosomes that segregate independently. Therefore, disparities may occur between the donor killer immunoglobulin-like receptors and the recipient HLA-KIR ligands. This may allow donor NK cells to become alloreactive against recipient cells that lack corresponding killer immunoglobulin-like receptor ligands.

Recipient class 1 major histocompatibility complex expression may be down-regulated after infection by many viruses and NK cell inhibitory receptors may be important to monitor for such infections. NK cell activation after interaction with virally infected cells may occur from either an increased avidity for such targeted cells or from a down-modulation of ligands for inhibitory receptors.[28]

Cook and associates reported that recipients of matched sibling donor myeloablative allogeneic hematopoietic stem cell transplant whose donors had more than one activating killer immunoglobulin-like receptor gene had a 65% reduction in *cytomegalovirus* reactivation[16] This protective effect was not observed for their reduced-intensity conditioning allogeneic hematopoietic stem cell transplant patients who had received lymphoid depletion with alemtuzumab. Chen and associates also found that additional activating killer immunoglobulin-like receptor genes in the donor were associated with a lower risk of

cytomegalovirus reactivation for recipients of myeloablative allogeneic hematopoietic stem cell transplant.[17]

Zai and associates observed less *cytomegalovirus* reactivation in patients whose donors had 5 or more activating killer immunoglobulin-like receptor genes than those with fewer numbers.[18] They also reported that the presence of a donor activating KIR2DS2 or KIR2DS4 predicted a lower risk of *cytomegalovirus* reactivation. The majority of patients in this report received myeloablative allogeneic hematopoietic stem cell transplant and approximately one-third had unrelated donors.

This report demonstrates that patients with donors having 5 to 6 as compared to 1 to 4 activating killer immunoglobulin-like receptor genes had significantly less *cytomegalovirus* reactivation after T-cell replete, matched sibling donor reduced-intensity conditioning allogeneic hematopoietic stem cell transplant. In contrast to the report from Zai and associates, we did not find a specific activating killer immunoglobulin-like receptor gene to be associated with *cytomegalovirus* reactivation. However, our results are in agreement with their report in that no difference in *cytomegalovirus* reactivation was observed when assessing inhibitory killer immunoglobulin-like receptor genotypes. We did not observe a difference in *cytomegalovirus* reactivation for patients whose donors had a killer immunoglobulin-like receptor A/A genotype as compared to those with killer immunoglobulin-like receptor B/X genotype.

Although the group with 1 to 4 activating killer immunoglobulin-like receptor genes had some cases of grade 2 to 4 acute graft-versus-host disease before *cytomegalovirus* reactivation, the majority of these patients did not, and this was not significant on multivariable analyses. There also were no differences in grades 3 to 4 acute graft-versus-host disease or chronic graft-versus-host disease between those whose donors had 1 to 4 or 5 to 6 activating killer immunoglobulin-like receptor genes. The finding of higher rates of *cytomegalovirus* reactivation for patients who received 200 cGy as compared to 400 cGy total body irradiation was unexpected. It may be possible that those who received 400 cGy had more rapid immune reconstitution posttransplant.

Assessment of donor killer immunoglobulin-like receptor genotype may have important implications for predicting *cytomegalovirus* reactivation after T-cell replete, matched sibling donor reduced-intensity conditioning allogeneic hematopoietic stem cell transplant. These results may help guide selection of donors and identify patients who may benefit from closer *cytomegalovirus* monitoring and additional strategies to prevent *cytomegalovirus* reactivation posttransplant.

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