Brief report

Donor *KIR* genotype has a major influence on the rate of cytomegalovirus reactivation following T-cell replete stem cell transplantation

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Reactivation of cytomegalovirus (CMV) is a common complication following allogeneic stem cell transplantation. Genetic determinants in the host and donor that may influence the rate of reactivation are currently unknown. Viral replication is controlled by T cells and natural killer (NK) cells and these share expression of killer immunoglobulin-like receptors (KIRs). We analyzed whether activatory KIRs carried by the donor influenced the subsequent rate of CMV reactivation in the patient. In transplantations involving siblings where both donor and recipient were CMV seropositive, donors with more than one activating *KIR* gene were associated with a 65% reduction in CMV reactivation. Multivariate analysis confirmed a significantly reduced risk of CMV reactivation in sibling transplantations where the donor had more than one activating KIR. Reduced-intensity transplantation and graft-versus-host disease grade 2 or higher were associated with an increased risk of CMV reactivation. This observation indicates that activating KIRs play an important role in the cellular control of CMV reactivation. (Blood. 2006;107: 1230-1232)

Introduction

Cytomegalovirus (CMV) reactivation is the most common viral complication following allogeneic hematopoietic stem cell transplantation (HSCT).[1] Interest in the use of cellular immunotherapy to control viral reactivation is increasing and it is important to understand the immune mechanisms by which viral replication is controlled. Natural killer (NK) cells and T cells are the primary effector populations that suppress CMV replication and CMV encodes several proteins that interact with NK and T-cell molecules to facilitate immune evasion.[2]

Killer immunoglobulin-like receptors (KIRs) are expressed on the surface of NK cells and T-cell subsets.[3-5] Inhibitory KIRs deliver an inhibitory signal to the cell. They bind HLA class I molecules and individual KIRs have specificity for defined alleles of HLA-C, HLA-B, or HLA-A.[3] Activating KIRs, which deliver an activating signal, bind only weakly to these HLA molecules[6,7] and their natural ligands remain undetermined. *KIR* genes are highly polymorphic and there is variation in the number of inherited *KIR* genes.[8] Two broad haplotypes of *KIR* genes have been defined. In addition to the so-called "framework loci" common to both haplotypes, the A haplotype most commonly carries a single activating *KIR* gene, *KIR2DS4*. The B haplotype is characterized by additional activating *KIR* loci and the inhibitory KIR *KIR2DL5*.[8,9] Approximately 35% of the white population is homozygous for the A haplotype. The remainder carries at least one B haplotype and therefore potentially expresses multiple activating KIRs. The biologic factors maintaining this degree of polymorphism within the population are unknown.

Study design

We hypothesized that the KIR repertoire of the donor might influence the probability of CMV reactivation after HSCT. The donor *KIR* genotype and history of CMV reactivation were assessed in 234 patients following HSCT for myeloid malignancy (n = 97), lymphoid malignancy (n = 87), and nonmalignant disease (n = 50) between October 1999 and November 2002 at 3 institutions in the United Kingdom. Donor cells were transplanted either without manipulation of the stem cell graft or with a T-cell depletion step using alemtuzumab for unrelated donor, haploidentical donor, or as part of a reduced-intensity regimen. CMV serostatus was determined by enzyme-linked immunosorbent assay (ELISA; VIDAS-bioMerieux, Marcy L'Etoile, France). Posttransplant monitoring for CMV reactivation was performed by polymerase chain reaction (PCR; COBAS Amplicor CMV Monitor test, Roche Diagnostics, Pleasanton, CA). Reactivation was defined as 2 successive assays detecting more than 400 copies/mL. *KIR* genotyping was performed by PCR as previously described.[10] For statistical analysis, comparisons of frequencies were made using 2×2 contingency tables analyzed with χ^2 or Fisher exact test; actuarial data were analyzed by the log-rank method. The effect of variables (KIR haplotype group of donor, age of recipient at time of transplantation, CMV serostatus of recipient, CMV serostatus of donor, sex mismatch, disease type, myeloablative/

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Submitted March 15, 2005; accepted July 26, 2005. Prepublished online as *Blood* First Edition Paper, October 20, 2005; DOI 10.1182/blood-2005-03-1039.

Supported in part by a grant from the Howard Ostins Trust.

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reduced-intensity conditioning, graft-versus-host disease [GvHD] prophylaxis, and GvHD of grade 2 or greater requiring treatment) on time to CMV reactivation were tested in a Cox regression model using SPSS for Windows version 11.5 (SPSS, Chicago, IL). Informed consent was obtained for collection of samples and usage of data was obtained in accordance with the Declaration of Helsinki. The study was part of a research protocol approved by South Birmingham Research Ethics Committee.

Results and discussion

In CMV seropositive recipients the CMV reactivation rate was 53% in transplants from sibling donors (38 of 72) and 64% in transplants from unrelated or HLA nonidentical donors (22 of 35, *P* was not significant). The rate of CMV reactivation in the seronegative patient group (n = 127) was less than 4% and these were not studied further.

In transplantations involving siblings where both donor and recipient were CMV seropositive and the donor was homozygous for *KIR* haplotype "A" (termed group A donors), the CMV reactivation rate was 65% (15 of 23). In contrast, if the donor possessed a copy of the *KIR* haplotype B (termed group B donors), the reactivation rate was 28% (8 of 29, $P = .014$). This protective influence of group B donors appeared restricted to patients who received a myeloablative stem cell transplant. Here, the reactivation rate was 71% (12 from 17) when transplanted from a group A donor compared with 24% (5 from 21) when transplanted from a group B donor ($P = .001$). No reactivation was seen after 41 days in the transplants from group B donors, whereas reactivation continued up to day 100 in transplants from group A donors (Figure 1 and Table 1, $P = .018$). On multivariate analysis, group B donors significantly reduced the rate of reactivation in transplantations involving siblings (RR 0.308 [95% CI 0.131-0.724], $P = .007$). An increased rate of reactivation was associated with recipient CMV seropositive (RR 12.56 [95% CI 3.48-45.35], $P < .001$), reduced-intensity transplant (RR 4.032 [95% CI 1.51-10.78], $P = .007$), and GvHD of grade 2 or greater (RR 2.62 [95% CI 1.06-6.45] $P = .037$). No such effects were seen in either volunteer unrelated donor (VUD) or haploidentical transplantations.

This study demonstrates that, in transplantations involving siblings, the protective effect of transfer of CMV-specific immunity from donor to recipient depends on the *KIR* genotype of the donor. This benefit was not seen in reduced-intensity transplants that involved lymphoid depletion using alemtuzumab. Similarly, no such effect was seen in VUD or haploidentical transplants, both of which used extensive lymphoid depletion. The protective influence of activating KIRs, therefore, appears to require the presence of donor lymphocytes at the time of transplantation.

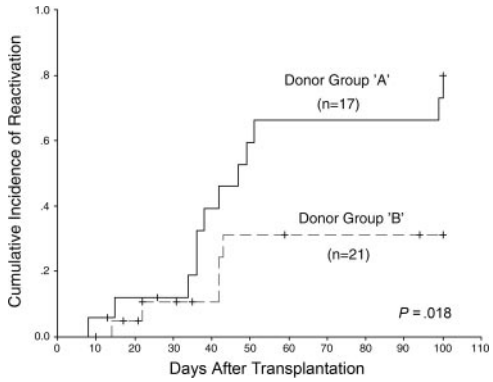

Figure 1. Rate of CMV reactivation. Donors with the KIR 'B' haplotype significantly reduce the rate of CMV reactivation in sibling allogeneic HSCT (n = 38).

Table 1. Patient characteristics according to the donor KIR type

	Donor group A	Donor group B
No. patients	80	154
Recipient age, y (range)	31.5 (1-60)	32.7 (1-59)*
Donor type		
Sibling	50	95*
VUD	23	42*
Haploidentical	7	17*
Sex mismatch	37	77*
Malignant disease	70	132*
Reduced-intensity conditioning	15	42*
GvHD prophylaxis		
CSA	27	57*
CSA/MTX	31	55*
CSA/alemtuzumab with or without MTX	22	42*
Recipient CMV^+	51	104*
Donor CMV^+	42	69*
Grade 2 or higher GvHD	17	42*
Prior immunosuppression	36	76*
Advanced/poor-risk disease	25	63*

CSA, indicates cyclosporin A; MTX, methotrexate
*P was not significant (>.05).

The mechanism behind this protective effect of activating KIRs is uncertain. The true ligands for activating KIRs remain to be determined. Although considerable homology exists between the extracellular sequence of activating and inhibitory KIRs suggesting that they share ligands, activating KIRs demonstrate only a weak affinity for class I HLA. The interaction between activating KIRs and class I HLA may be dependent on the peptide presented within the peptide binding groove of the class I HLA molecule because there is evidence that peptides affect the interaction between inhibitory KIRs and class I HLA.[6,11,12] Alternatively, the true ligands for activating KIRs may not be class I HLA molecules but virally encoded homologues. In mice, the lectin-type activating receptor Ly49H confers resistance against murine CMV by engaging a major histocompatibility (MHC)–like protein encoded by the virus.[13] In humans the KIR-related protein LILRB1 binds to the CMV-encoded HLA class I homologue UL18 and shows increased expression on lymphocytes in lung transplant patients with CMV disease[14] and there is evidence that CMV seropositivity influences the cell surface expression of molecules encoded within the leukocyte receptor complex (LRC).[15] Inhibitory KIRs have recently been shown to influence the rate of clearance of hepatitis C[16] using a genetics approach to study affected individuals.

It is now believed that CMV undergoes intermittent reactivation in immunocompetent hosts. If activating KIRs also play a significant role in controlling CMV reactivation in healthy donors, this may contribute to the maintenance of their polymorphism within the population. Any potential benefit of inheriting multiple activating KIRs must be weighed against their potential contribution toward other disease. Indeed, the inheritance of activating KIRs in the absence of an inhibitory homologue has been shown to increase the risk of psoriatic arthritis and other autoimmune phenomena.[17,18]

Using a genetics approach we have identified a novel interaction between host genotype and CMV reactivation. This is the first study to show that the inheritance of multiple activating *KIR* genes can influence the immune control of viral replication. Although we have studied a transplantation setting associated with a high rate of viral reactivation, this phenomenon may be more generally applicable and provide a novel insight into viral immunity. In the setting of HSCT this observation may allow improved donor selection and guide appropriate cellular immunotherapy to reduce the incidence of viral reactivation.

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