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

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Running Head Donor KIR Genotype and CMV Reactivation
Word Count
Abstract 150
Text 1207

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

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 NK cells and these share expression of killer immunoglobulin-like receptors (KIRs). We analysed whether activatory KIRs carried by the donor influenced the subsequent rate of CMV reactivation in the patient. In sibling transplants 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 transplants where the donor had more than one activating KIR. Reduced-intensity transplant and GvHD > grade 2 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.
Cytomegalovirus (CMV) reactivation is the commonest viral complication following allogeneic haematopoietic stem cell transplantation (HSCT). 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 which suppress CMV replication and CMV encodes several proteins which interact with NK and T-cell molecules to facilitate immune evasion. Killer immunoglobulin-like receptors (KIRs) are expressed on the surface of NK cells and T-cell subsets. 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. Activating KIRs, which deliver an activating signal, bind only weakly to these HLA molecules and their natural ligands remain undetermined. KIR genes are highly polymorphic and there is variation in the number of inherited KIR genes. 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 characterised by additional activating KIR loci and the inhibitory KIR KIR2DL5. Approximately 35% of the Caucasoid population is homozygous for the A haplotype. The remainder carries at least one B haplotype and therefore potentially expresses multiple activating KIRs. The biological factors maintaining this degree of polymorphism within the population are unknown.

Study Design

We hypothesised 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 non-malignant disease (n=50) between October 1999 and November 2002 at three UK institutions. Donor cells were transplanted either without manipulation of the stem cell graft or with a T-cell depletion step using Campath-1H for unrelated donor, haploidentical donor or as part of a reduced-intensity regimen. CMV sero-status was determined by ELISA (VIDAS- bioMerieux SA, France). Post-transplant monitoring for CMV reactivation was performed by PCR (COBAS Amplicor CMV Monitor test, Roche Diagnostics, Pleasanton, California). Reactivation was defined as two successive assays detecting >400 copies/ml. KIR genotyping was performed by PCR as previously described. For statistical analysis, comparisons of frequencies were made using 2x2 contingency tables analysed with chi-squared or Fisher’s exact test, actuarial data was analysed by the log-rank method. The effect of variables (KIR haplotype group of donor, age of recipient at time of transplant, CMV serostatus of recipient, CMV serostatus of donor, sex mismatch, disease type,
myeloablative/reduced intensity conditioning, 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 Inc., Chicago, Illinois). Informed consent was obtained for collection of samples and usage of data. 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 out of 72) and 64% in transplants from unrelated or HLA non-identical donors (22 out of 35, p=ns). The rate of CMV reactivation in the sero-negative patient group (n=127) was found to be less than 4% and these were not studied further.

In sibling transplants 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 out 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 out of 29, p=0.014). 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=0.0039). 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, p=0.018). On multivariate analysis, group B donors significantly reduced the rate of reactivation in sibling transplants (RR 0.308 (95%CI 0.131-0.724, p=0.007)). An increased rate of reactivation was associated with recipient CMV seropositive (RR 12.56 (95%CI 3.48-45.35, p<0.0001), reduced intensity transplant (RR 4.032 (95%CI 1.51-10.78, p=0.007)) and GvHD of grade 2 or greater (RR 2.62 (95%CI 1.06-6.45, p=0.037). No such effects were seen in either VUD or haploidentical transplants.

This study demonstrates that, in sibling transplants, the protective effect of transfer of CMV-specific immunity from donor to recipient is dependent on the KIR genotype of the donor. This benefit was not seen in reduced intensity transplants that involve lymphoid depletion using Campath-1H. 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.

The mechanism behind this protective effect of activating KIRs is uncertain. The true ligands for activating KIRs remain to be determined. Whilst there is considerable homology 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 as there is evidence that peptides affect the interaction between inhibitory KIRs and class I HLA\textsuperscript{6,11,12}. Alternatively, the true ligand(s) 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\textsuperscript{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\textsuperscript{14} and there is evidence that CMV seropositivity influences the cell surface expression of molecules encoded within the leukocyte receptor complex (LRC)\textsuperscript{15}. Inhibitory KIRs have recently been shown to influence the rate of clearance of hepatitis C\textsuperscript{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 towards 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\textsuperscript{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 transplant 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 haematopoietic stem cell transplantation this observation may allow improved donor selection and guide appropriate cellular immunotherapy to reduce the incidence of viral reactivation.
References

Figure 1. 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.

*p=NS (>0.05)

CSA= Ciclosporin A, MTX= Methotrexate

<table>
<thead>
<tr>
<th></th>
<th>Donor Group A (n=80)</th>
<th>Donor Group B (n=154)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient Age in Years (Range)</td>
<td>31.5 (1-60)</td>
<td>32.7 (1-59)*</td>
</tr>
<tr>
<td>Donor Type</td>
<td></td>
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<tr>
<td>Sibling</td>
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<td>95*</td>
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<tr>
<td>VUD</td>
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<td>42*</td>
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<tr>
<td>Haploidentical</td>
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<td>17*</td>
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<tr>
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<td>77*</td>
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<tr>
<td>Malignant Disease</td>
<td>70</td>
<td>132*</td>
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<tr>
<td>Reduced Intensity Conditioning</td>
<td>15</td>
<td>42*</td>
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<tr>
<td>GvHD Prophylaxis</td>
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<td></td>
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<tr>
<td>CSA</td>
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<tr>
<td>CSA/MTX</td>
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<td>55*</td>
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<td>CSA/Campath +/- MTX</td>
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<tr>
<td>Recipient CMV positive</td>
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<td>104*</td>
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<tr>
<td>Donor CMV positive</td>
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<td>76*</td>
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<tr>
<td>Advanced/Poor-Risk Disease</td>
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<td>63*</td>
</tr>
</tbody>
</table>
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