Brief report

Missing KIR ligands are associated with less relapse and increased graft-versus-host disease (GVHD) following unrelated donor allogeneic HCT

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Natural killer (NK) cells can alter the outcome of hematopoietic cell transplantation (HCT) if donor alloreactivity targets the recipient. Since most NK cells express inhibitory killer-immunoglobulin receptors (KIRs), we hypothesized that the susceptibility of recipient cells to donor NK cell–mediated lysis is genetically predetermined by the absence of known KIR ligands. We analyzed data from 2062 patients undergoing unrelated donor HCT for acute myeloid leukemia (AML; n = 556), chronic myeloid leukemia (CML; n = 1224), and myelodysplastic syndrome (MDS; n = 282). Missing 1 or more KIR ligands versus the presence of all ligands protected against relapse in patients with early myeloid leukemia (relative risk [RR] = 0.54; n = 536, 95% confidence interval [CI] 0.30-0.95, *P* = .03). In the subset of CML patients that received a transplant beyond 1 year from diagnosis (n = 479), missing a KIR ligand independently predicted a greater risk of developing grade 3-4 acute graft-versus-host disease (GVHD; RR = 1.58, 95% CI 1.13-2.22; *P* = .008). These data support a genetically determined role for NK cells following unrelated HCT in myeloid leukemia. (Blood. 2007;109:5058-5061)

Introduction

Natural killer (NK) cells display a range of receptors that ensure tolerance to healthy cells and lytic responses to diseased and allogeneic cells.[1-5] Among these are the killer-immunoglobulin receptors (KIRs), a diverse family of clonally restricted activating or inhibitory receptors. NK cells that display a given inhibitory KIR cannot respond to cells carrying a cognate HLA class I molecule, also called the KIR ligand.[6] Several investigations, mainly those using T-cell depletion (TCD), have shown that following allogeneic hematopoietic cell transplantation (HCT), KIR ligand status predicts donor antirecipient alloreactivity, which can prevent relapse and reduce graft-versus-host disease (GVHD).[7-10]

Clonal analysis has shown that each NK cell expresses an inhibitory receptor for autologous HLA class I, either a KIR or the HLA-E–specific CD94:NKG2A heterodimer.[11] Consequently, an individual's NK-cell repertoire is functionally dependent upon both the *KIR* and *HLA* genes. Beneficial alloreactive NK-cell responses are detectable for only a few months following haploidentical transplantation.[12] During that time the NK-cell repertoire appears unconstrained by recipient HLA; thereafter it becomes tolerant of the recipient HLA type, presumably in part through expression of inhibitory KIR. NK-cell alloreactivity can be observed in HLA-matched HCT,[13] where the influence of HLA on early reconstituting NK cells is temporarily suspended, such that "forbidden clones" arise with alloreactivity toward the recipient's HLA type.

Most humans have inhibitory KIRs specific for C1 (HLA-C alleles with Ser77, Asn80), C2 (HLA-C alleles with Asn77, Lys80), and Bw4. For example, in white populations the frequencies are *KIR2DL1* (91%-100%), *KIR2DL2* (49%-60%), *KIR2DL3* (85%-93%), and *KIR3DL1* (87%-98%).[14] Consequently, most donor-derived NK cells express inhibitory KIRs. Therefore, NK-cell alloreactivity after HCT might simply correlate with the number and type of KIR ligands a recipient lacks.

Results and discussion

We studied 2062 patients (556 with acute myeloid leukemia [AML], 1224 with chronic myeloid leukemia [CML], and 282 with myelodysplastic syndrome [MDS]) whose transplantations were facilitated through the National Marrow Donor Program (NMDP) between 1988 and 2000. All patient samples and data were collected after obtaining informed consent, in accordance with the Declaration of Helsinki, using NMDP database and Sample Repository institutional review board (IRB)–approved protocols. Presence or absence of recipient C1, C2, and Bw4 KIR ligands was determined based on high-resolution HLA typing confirmed using blood samples collected and stored by

Submitted January 8, 2007; accepted February 12, 2007. Prepublished online as *Blood* First Edition Paper, February 22, 2007; DOI 10.1182/blood-2007-01-065383.

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the NMDP at their Research Sample Repository. All patients had either 1, 2, or 3 KIR ligands and, as there was no dose effect with the number of missing ligands, ligand absence was analyzed as a group. A first analysis of all the patients revealed no differences in relapse or survival based on KIR ligand content. When the patients were stratified according to disease stage, significant differences emerged. For patients with early disease (AML in first complete response [CR1], CML in first chronic phase and less than 1 year from diagnosis, or MDS with refractory anemia), the absence of 1 or more KIR ligands was protective against relapse (relative risk [RR] 0.54, n = 536, 95% confidence interval [CI] 0.30-0.95, $P = .03$; Table 1). This finding was independent of HLA matching and independent of TCD, consistent with the observed beneficial NK cell–mediated effects in transplantation with HLA-matched sibling donors.[13] Univariate analysis showed a decrease in relapse at 3 years from 11% (CI: 7%-17%) to 6% (CI: 4%-9%). In contrast, the absence of 1 or more KIR ligands did not affect clinical outcomes for patients with more advanced myeloid disease. HLA matching and a diagnosis of CML were the predominant factors that predicted better survival in this group, and missing KIR ligands did not influence survival.

Host antigen-presenting cells (APCs) play an important role in GVHD through the presentation of alloantigen to donor T cells.[9] NK-cell killing of host dendritic cells has the potential to interrupt GVHD, and we have recently shown that interferon-γ production by NK cells correlates with acute GVHD (aGVHD) incidence in T-cell replete transplantation.[15] Since these results suggest that NK cells may play an indirect role in GVHD, we analyzed aGVHD severity based on the presence or absence of KIR ligands. HLA matching and TCD decreased the risk of aGVHD as expected. Missing 1 or more KIR ligands had no independent effect on aGVHD except in patients with CML more than 1 year from diagnosis where it was associated with an unexpected high incidence of grade 3-4 aGVHD (RR 1.58, n = 479, 95% CI 1.13-2.22, $P = .008$; Table 2). This corresponded to an increase in severe aGVHD from 30% (CI: 23%-37%) for patients with all KIR ligands present to a rate of 44% (CI: 39%-50%) for patients missing 1 or more KIR ligands. We had hypothesized that potentially alloreactive NK cells would be expected to kill host dendritic cells, thus decreasing the severity of aGVHD.[7] In our cohort, perhaps T cells in the graft were active immediately after transplantation, establishing aGVHD before NK cells had the opportunity to kill host APCs.

Interest in the role of NK cells in KIR ligand–mismatched transplantations has led to many reports with mixed conclusions that are difficult to reconcile.[7,8,10,16-19] Several studies show benefit, which may correlate with the extent to which the graft is depleted of T cells.[15] Because most individuals have inhibitory KIRs for HLA-C1, C2, and Bw4, we explored a model based on recipient HLA typing alone to determine KIR ligand status. Our results suggest that early stage myeloid leukemia patients relapse less if they are missing a Bw4, C1, or C2 ligand. This finding became apparent only when disease stage was examined independently, likely because patients with advanced leukemia do less well overall. The effect of missing KIR ligands in patients with early leukemia may reflect the alloreactivity of donor-derived NK cells that are not inhibited by recipient HLA class I, although the divergence of effects between patients with early or advanced disease may implicate other mechanisms. For example, T-cell

Table 1. Multivariate analysis of relapse following unrelated donor HCT

Variable	Cohort size, no.	Relative risk (95% CI)	*P*
Early: AML CR1, CML with CP1 less than 1 y from diagnosis, and MDS (RA)	536		
Recipient ligand status			
All KIR-L present	182	1.00 (reference)	—
Missing 1 or more KIR-L	354	0.54 (0.30-0.95)	.03
Disease			
AML	95	1.00 (reference)	—
CML	372	0.23 (0.12-0.42)	< .001
MDS	69	0.49 (0.21-1.17)	.11
Early CML chronic phase more than 1 y from diagnosis	481		
Recipient ligand status			
All KIR-L present	171	1.00 (reference)	—
Missing 1 or more KIR-L	310	1.06 (0.51-2.21)	.88
Intermediate: AML more than CR1 or first relapse, CML more than CP1 or AP, MDS no excess blasts	706		
Recipient ligand status			
All KIR-L present	248	1.00 (reference)	—
Missing 1 or more KIR-L	458	0.80 (0.57-1.13)	.21
Disease			
AML	307	1.00 (reference)	—
CML	303	0.76 (0.53-1.08)	.13
MDS	96	0.37 (0.19-0.71)	.003
Advanced: AML in relapse, CML BC, MDS (RAEB/RAEBT)	339		
Recipient ligand status			
All KIR-L present	118	1.00 (reference)	—
Missing 1 or more 1 KIR-L	221	1.30 (0.83-2.04)	.26
Disease			
AML	154	1.00 (reference)	—
CML	68	0.77 (0.46-1.27)	.31
MDS	117	0.28 (0.16-0.49)	< .001

Regression models were adjusted for recipient and donor age, sex, year of transplantation, cytomegalovirus status, HLA matching, GVHD prophylaxis, and pretransplantation performance status. Ninety-eight percent of patients received traditional fully ablative preparative regimens; 79% received cyclophosphamide and total body irradiation; and 19% received busulfan and cyclophosphamide. There were no differences in the use of preparative regimens by disease stage.

CP indicates chronic phase; RA, refractory anemia; KIR-L, killer immunoglobulin receptor ligand (Bw4, C1 or C2 group); AP, accelerated phase; BC, blast crisis; and —, reference value.

Table 2. Analysis for acute grade 3-4 GVHD

Variable	Cohort size	Relative risk (95% CI)	*P*
Early: AML CR1, CML with CP1 less than 1 y from diagnosis, and MDS (RA)	534		
Recip ligand status			
All KIR-L present	181	1.00 (reference)	—
Missing 1 or more KIR-L	353	0.95 (0.68-1.33)	.75
HLA match status			
KIR-L MM (GVH)	44	1.00 (reference)	—
HLA MM only	188	1.00 (0.57-1.75)	.99
HLA matched	302	0.54 (0.31-0.94)	.03
Early CML chronic phase more than 1 y from diagnosis	479		
Recip ligand status			
All KIR-L present	170	1.00 (reference)	—
Missing 1 or more KIR-L	309	1.58 (1.13-2.22)	.008
HLA match status			
KIR-L MM (GVH)	60	1.00 (reference)	—
HLA MM only	209	0.96 (0.63-1.47)	.86
HLA matched	210	0.52 (0.34-0.80)	.003
ATG/TCD status			
ATG given and TCD	26	1.00 (reference)	—
ATG but no TCD	21	1.50 (0.54-4.16)	.43
No ATG but TCD	68	0.65 (0.26-1.66)	.37
No ATG or TCD	364	2.23 (1.04-4.78)	.04
Intermediate: AML more than CR1 or first relapse, CML more than CP1 or AP, MDS no excess blasts	702		
Recip ligand status			
All KIR-L present	246	1.00 (reference)	—
Missing 1 or more KIR-L	456	1.19 (0.89-1.59)	.24
HLA match status			
KIR-L MM (GVH)	71	1.00 (reference)	—
HLA MM only	312	0.97 (0.64-1.48)	.89
HLA matched	319	0.68 (0.44-1.03)	.07
Advanced: AML in relapse, CML BC, MDS (RAEB/RAEBT)	338		
Recip ligand status			
All KIR-L present	118	1.00 (reference)	—
Missing 1 or more KIR-L	220	0.75 (0.53-1.07)	.11

HLA match represents antigen level match at HLA-A, B and allele match at DRB1. The regression adjustments applied were the same as in Table 1.
KIR-L MM (GVH) indicates KIR ligand mismatch in GVH direction; and MM, mismatch. Additional abbreviations are explained in Table 1.

responses arising from peptide presentation by Bw4, C1, or C2 epitopes may be more important than NK-cell alloreactivity in transplantation for advanced disease. Additionally, NK-cell and T-cell effector function may reflect an interactive continuum between lymphocyte populations.

The increased aGVHD seen in patients with CML more than 1 year from diagnosis has not been reported previously and suggests an indirect role for NK cells in GVHD. This patient cohort, which predates the routine use of imatinib, was unique in that more advanced CML patients were treated with interferon compared with early chronic-phase patients (60% [n = 481] vs 41% [n = 372]; $P < .001$). However, when prior interferon therapy was analyzed with disease stage, no independent affect on aGVHD risk was seen. The higher rate of aGVHD in the CML cohort may be explained by the expanded myeloid pool with more host APCs capable of presenting alloantigen to donor T cells.

This study analyzed a large number of transplantations and identified 2 effects of missing KIR ligands on HCT outcome. This highlights the fact that KIR interactions are complicated and that their immune effects may compete with the effects of T cells or other immune effectors. Furthermore, the use of different GVHD prophylaxis and preparative regimens[7,8,16,19] (eg, potent T-cell depletion vs more traditional immunosuppression) may influence NK-cell recovery and function and explain different clinical outcomes. In summary, we report an apparent genetic predisposition to NK cell–mediated effects in standard-risk myeloid leukemia patients where absence of an HLA-B or HLA-C KIR ligand modifies specific clinical outcomes. Better understanding of how KIR repertoires can be manipulated in transplantation may facilitate further clinical benefit, perhaps through attenuation of KIR inhibitory signals.

Acknowledgments

This work was supported in part by National Institutes of Health Grant P01-CA-111412 to the University of Minnesota as well as Health Resources and Services Administration grant 240-97-0036 and Office of Naval Research grant N00014-99-2-0006 to the National Marrow Donor Program.

Any opinions, findings, and conclusion or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the Office of Naval Research, Defense Department, or the US government.

Authorship

Contribution: J.S.M. and D.J.W. created the study plan, performed data analysis, and prepared the manuscript; S.C., P.P., S.S.F., M.R.V., K.L.M., L.A.G., M.M.H., and J.P.K. performed data analysis and interpretation and prepared the manuscript; E.A.T. performed data interpretation and prepared the manuscript; and M.H. performed data analysis.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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