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KIR and HLA genotypes have no identifiable role in single unit dominance following double unit umbilical cord blood transplantation

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Umbilical cord blood (CB) is widely used as an alternative hematopoietic stem cell source. The use of double unit CB grafts is a common strategy to augment graft cell dose and successfully leads to engraftment by a single unit.[1, 2] Current evidence suggests unit dominance is T-cell mediated;[3-5] a role for natural killer (NK) cells, however, is not excluded. NK cells mediate murine bone marrow allograft rejection and are the first lymphocytes to reconstitute following CB transplantation (CBT).[6, 7] In addition, CB NK cells are of a mature phenotype and have high cytotoxic and proliferative capacity.[6, 8, 9] We, therefore, tested the hypothesis that unit dominance after double unit CBT results from an immune interaction mediated by NK cells between the two CB units or between the recipient and the infused units.

This is a retrospective analysis of 83 recipients of double unit CBT treated at Memorial Sloan-Kettering Cancer Center (MSKCC) between October 2005 and July 2010 for hematological malignancies [46 (55.4%) acute leukemia, 32 (38.5%) lymphoma and 5 (6%) other]. Sixty-six (80%) patients received a myeloablative conditioning regimen. All patients received immunosuppression with a calcineurin-inhibitor and mycophenolate mofetil. The median age of patients was 35.6 years (0.9-64.6). Informed consent for specimen collection was obtained from the patients or legal guardians in accordance with MSKCC review board guidelines. CB unit dominance was determined by molecular testing for donor chimerism

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from bone marrow and/or peripheral blood samples obtained at serial time-points starting from day+21 through 1 year following CBT. Unit dominance was established for all patients by day+100. Killer cell immunoglobulin-like receptors (KIR) genotyping was performed on genomic DNA[10] extracted from peripheral blood mononuclear cells or CB mononuclear cells using the QIAamp DNA Blood Minikit (Qiagen, Valencia, CA). HLA allele identification services were provided by the American Red Cross (Philadelphia, PA).

The frequency distributions of KIR haplotypes and activating KIR were calculated and a chi-square test was used to determine whether these distributions differed between dominant and non-dominant units. The frequency distributions for the dominant and non-dominant units were calculated and a chi-square test was used to test whether these distributions differed in unit-unit interaction and unit-recipient interaction. The statistical package SAS (9.2) was used to generate the test statistics.

KIR genotypes and HLA class I determinants of the infused CB units and the recipients are summarized in Table 1. KIR genotyping was available on 72 of the 83 CBT recipients. Results are summarized in Table 2. We compared the frequency distribution of AA and BX haplotypes within CB units. We investigated whether a CB unit exhibiting a B haplotype has more chances of dominance and investigated the impact of centromeric and telomeric B motifs in single unit dominance. CB units with A and B haplotypes showed the same rate of dominance and CB units with specific telomeric or centromeric haplotype B motifs did not show a dominance advantage. We then analyzed the frequency distribution of each activating KIR in both units. CB units possessing any one of the activating KIR did not show a higher chance of dominance. In addition, units lacking all activating KIR were not less likely to dominate. Of the activating KIR, only KIR2DS1 has class I specificity; we could not demonstrate that KIR2DS1 positive units with an HLA-C1 background were more likely to dominate if the second unit possessed HLA-C2.

According to the inhibitory KIR genotype and the presence or absence of HLA class I ligands in the CB units and the recipients, different KIR and HLA interactions predictive of a “missing ligand” or “missing self” effect were evaluated. To determine the role of HLA and KIR genotypes in unit-unit interaction and single unit dominance, we investigated the hypothesis that a CB unit fails to dominate if it lacks HLA class I ligand for an individual inhibitory KIR present in the second unit (missing ligand) or if it lacks HLA class I determinants present in the second unit (missing self). We divided the CB unit pairs into 4 categories depending if none, one, or both units are missing HLA KIR ligands or self-antigens for the other unit's inhibitory KIR.KIR-HLA interactions predictive of NK allo-reactivity between CB units in a unidirectional manner did not predict unit dominance. To determine the role of HLA and KIR genotypes in unit-recipient interaction and single unit dominance we investigated two different hypotheses: 1) a CB unit is more likely to dominate if the recipient lacks HLA class I ligand for specific inhibitory KIR present in the unit (missing ligand)or lacks HLA class I determinants present in the unit (missing self); or 2) a CB unit is less likely to dominate if it lacks HLA class I ligand for specific inhibitory KIR present in the recipient (missing ligand)or lacks HLA class I determinants present in the recipient (missing self). The CB unit pairs were divided in different categories depending if the recipient lacked HLA ligands or self-antigens for neither, one, or both units' inhibitory

KIR; or if neither, one or both units lacked HLA ligand or self-antigens for the recipient's inhibitory KIR. A chi-square test for equal distribution demonstrated that CB units did not have a higher likelihood of dominance if the patient was missing ligand or lacked class I determinants present in either unit. Similarly, CB units missing KIR ligand or lacking class I determinants present in the recipient did not have a lower rate of dominance.

In this cohort of 83 double unit CBT recipients, we failed to find an association between KIR and HLA genotypes with unit dominance. However, our small sample size and the limited number of CB pairs with unilateral NK alloreactivity may have precluded us in finding an association if one exists. The role of KIR and HLA genotypes in unit dominance still cannot be excluded and larger studies investigating this question are warranted.

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References

1. Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, McGlave PB, Miller JS, et al. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood. 2005; 105(3):1343–7. [PubMed: 15466923]
2. Kang HJ, Yoo KH, Lee JW, Kim H, Lee SH, Sung KW, et al. Double umbilical cord blood transplantation for children and adolescents. Ann Hematol. 89(10):1035–44. [PubMed: 20508938]
3. Eldjerou LK, Chaudhury S, Baisre-de Leon A, He M, Arcila ME, Heller G, et al. An in vivo model of double-unit cord blood transplantation that correlates with clinical engraftment. Blood. 2010; 116(19):3999–4006. [PubMed: 20587781]
4. Avery S, Shi W, Lubin M, Gonzales AM, Heller G, Castro-Malaspina H, et al. Influence of infused cell dose and HLA match on engraftment after double-unit cord blood allografts. Blood. 2011; 117(12):3277–85. quiz 3478. [PubMed: 21149633]
5. Gutman JA, Turtle CJ, Manley TJ, Heimfeld S, Bernstein ID, Riddell SR, et al. Single-unit dominance after double-unit umbilical cord blood transplantation coincides with a specific CD8+ T-cell response against the nonengrafted unit. Blood. 115(4):757–65. [PubMed: 19822900]
6. Beziat V, Nguyen S, Lapusan S, Hervier B, Dhedin N, Bories D, et al. Fully functional NK cells after unrelated cord blood transplantation. Leukemia. 2009; 23(4):721–8. [PubMed: 19151772]
7. Murphy WJ, Kumar V, Bennett M. Rejection of bone marrow allografts by mice with severe combined immune deficiency (SCID). Evidence that natural killer cells can mediate the specificity of marrow graft rejection. J Exp Med. 1987; 165(4):1212–7. [PubMed: 3549961]
8. Han P, Hodge G, Story C, Xu X. Phenotypic analysis of functional T-lymphocyte subtypes and natural killer cells in human cord blood: relevance to umbilical cord blood transplantation. Br J Haematol. 1995; 89(4):733–40. [PubMed: 7772509]
9. Woll PS, Martin CH, Miller JS, Kaufman DS. Human embryonic stem cell-derived NK cells acquire functional receptors and cytolytic activity. J Immunol. 2005; 175(8):5095–103. [PubMed: 16210613]
10. Hsu KC, Liu XR, Selvakumar A, Mickelson E, O'Reilly RJ, Dupont B. Killer Ig-like receptor haplotype analysis by gene content: evidence for genomic diversity with a minimum of six basic framework haplotypes, each with multiple subsets. J Immunol. 2002; 169(9):5118–29. [PubMed: 12391228]

Table 1
KIR and HLA genotypes for double unit CBT recipients and CB units

	Recipients	Dominant CB units	Non-dominant CB units
KIR Genotype	Total n=72	Total n=83	Total n=83
Activating KIR			
2DS1			
Present	29 (40%)	29 (35%)	25 (30%)
Absent	43 (60%)	54 (65%)	58 (70%)
2DS2			
Present	38 (53%)	42 (51%)	37 (45%)
Absent	34 (47%)	41 (49%)	46 (55%)
2DS3			
Present	22 (31%)	25 (30%)	24 (29%)
Absent	50 (69%)	58 (70%)	59 (71%)
2DS4			
Present	60 (83%)	55 (66%)	45 (54%)
Absent	12 (17%)	28 (34%)	38 (46%)
2DS5			
Present	23 (32%)	32 (38.5%)	30 (36%)
Absent	49 (68%)	51 (61.5%)	53 (64%)
3DS1			
Present	29 (40%)	30 (36%)	41 (49%)
Absent	43 (60%)	53 (64%)	42 (51%)
KIR 1D			
Present	33 (46%)	44 (53%)	44 (53%)
Absent	39 (54%)	39 (47%)	39 (47%)
Inhibitory KIR			
2DL1			
Present	67 (93%)	53 (64%)	47 (57%)
Absent	5 (7%)	30 (36%)	36 (43%)
2DL2			
Present	37 (51%)	44 (53%)	36 (43%)
Absent	35 (49%)	39 (47%)	47 (57%)
2DL3			
Present	61 (85%)	69 (83%)	75 (90%)
Absent	11 (15%)	14 (17%)	8 (10%)
3DL1			
Present	67 (93%)	79 (95%)	72 (87%)
Absent	5 (7%)	4 (5%)	11 (13%)
Haplotypes			

	Recipients	Dominant CB units	Non-dominant CB units
AA	22 (31%)	16 (19.3%)	22 (26.5%)
BX	50 (69%)	67 (80.7%)	61 (73.5%)
HLA KIR ligands	Total n=83	Total n=83	Total n=83
HLA-B			
Bw4/Bw4	16 (19%)	16 (19%)	16 (19%)
Bw4/Bw6	47 (57%)	34 (41%)	41 (50%)
Bw6/Bw6	20 (24%)	33 (40%)	26 (31%)
HLA-C			
C1/C1	27 (32%)	24 (29%)	27 (33%)
C1/C2	38 (46%)	34 (41%)	39 (47%)
C2/C2	18 (22%)	25 (30%)	17 (20%)

Table 2

KIR and HLA genotypes do not predict unit dominance following double unit CBT

KIR haplotype B and the presence of one or more activating KIR were not associated with CB unit dominance. KIR/HLA genotypes in unit-unit and unit-recipient interactions were not associated with CB unit dominance.

KIR HAPLOTYPES			
	Dominant CB units	**Non-dominant CB units**	**P value**
	Total n=83 n(%)	**Total n=83 n (%)**	
Centromeric KIR			
AA	38 (45.7%)	41(49.4%)	0.64
AB+BB	45 (54.3%)	42 (50.6%)	
Activating KIR			
None	9 (10.8%)	8 (9.6%)	0.80
>1	74 (89.2%)	75 (90.4%)	
KIR and HLA Genotypes			
		Observed N	**P value**
Unit-Unit interaction			
Non-dominant unit missing KIR ligand		13	0.85
Dominant unit missing KIR ligand		15	
Both units missing KIR ligand (n=35)			
Neither unit missing KIR ligand (n=20)			
Non-dominant unit missing self		6	0.23
Dominant unit missing self		12	
Both units missing self (n=24)			
Neither unit missing self (n=41)			
Unit-Recipient interaction			
Recipient missing ligand for dominant unit inhibitory KIR		5	0.45
Recipient missing ligand for non-dominant unit inhibitory KIR		2	
Recipient missing ligand for both units (n=43)			
Recipient missing ligand for neither units (n=33)			
Recipient missing self for dominant unit inhibitory KIR		9	0.8
Recipient missing self for non-dominant unit inhibitory KIR		7	
Recipient missing self for both units (n=9)			
Recipient missing self for neither units (n=58)			
Non-dominant unit missing ligand for recipient inhibitory KIR		11	0.83
Dominant unit missing ligand for recipient inhibitory KIR		13	
Both units missing KIR ligand (n=33)			
Neither unit missing KIR ligand (n=15)			

KIR HAPLOTYPES			
	Dominant CB units	**Non-dominant CB units**	**P value**
	Total n=83 n(%)	**Total n=83 n (%)**	
Non-dominant unit missing self for recipient inhibitory KIR	8		0.81
Dominant unit missing self for recipient inhibitory KIR	10		
Both units missing self (n=11)			
Neither unit missing self (n=43)			