

# *KIR2DS3* is Associated with Protection against Acute Myeloid Leukemia

Farhad Shahsavar<sup>1,2</sup>, Nader Tajik<sup>1\*</sup>, Kobra-Zinat Entezami<sup>1</sup>, Masoomeh Fallah Radjabzadeh<sup>1</sup>, Behnam Asadifar<sup>1</sup>, Kamran Alimoghaddam<sup>3</sup>, Mohammadreza Ostadali Dahaghi<sup>3</sup>, Arash Jalali<sup>3,4</sup>, Andisheh Ghashghaie<sup>3</sup>, Ardeshir Ghavamzadeh<sup>3</sup>

<sup>1</sup>Division of Transplant Immunology and Immunogenetics, Department of Immunology, Iran University of Medical Sciences, Tehran, Iran, <sup>2</sup>Department of Immunology, Lorestan University of Medical Sciences, Khorram Abad, Iran, <sup>3</sup>Hematology-Oncology and Stem Cell Transplantation Research Center, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran, <sup>4</sup>Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

## ABSTRACT

**Background:** Interaction between killer cell immunoglobulin-like receptors (KIR) and human leukocyte antigen (HLA) class I molecules is important for regulation of natural killer (NK) cell function. **Objective:** The aim of this study was to investigate the impact of compound KIR-HLA genotype on susceptibility to acute leukemia. **Methods:** Cohorts of Iranian patients with acute myeloid leukemia (AML; n=40) and acute lymphoid leukemia (ALL; n=38) were genotyped for seventeen *KIR* genes and their three major *HLA class I ligand* groups (*C1*, *C2*, *Bw4*) by a combined polymerase chain reaction–sequence-specific primers (PCR-SSP) assay. The results were compared with those of 200 healthy control individuals. **Results:** We found a significantly decreased frequency of *KIR2DS3* in AML patients compared to control group (12.5% vs. 38%, odds ratio=0.23, p=0.0018). Also, the *KIR3DS1* was less common in AML group than controls (27.5% vs. 44.5%, p=0.0465, not significant after correction). Other analyses including *KIR* genotypes, distribution and balance of inhibitory and activating *KIR+HLA* combinations, and co-inheritance of activating *KIR* genes with inhibitory *KIR+HLA* pairs were not significantly different between leukemia patients and the control group. However, in AML patients a trend toward less activating and more inhibitory *KIR-HLA* state was observed. Interestingly, this situation was not found in ALL patients and inhibition enhancement through increase of HLA ligands and inhibitory combinations was the main feature in this group. **Conclusion:** Our findings may suggest a mechanism for escape of leukemic cells from NK cell immunity.

**Keywords:** Acute Leukemia, Genotype, HLA, KIR

\*Corresponding author: Dr. Nader Tajik, Division of Transplant Immunology and Immunogenetics, Department of Immunology, Iran University of Medical Sciences, Tehran, Iran. Tel: (+) 98 912 3250344, Fax: (+) 98 21 88058652, e-mail: nadertajik@iums.ac.ir

## INTRODUCTION

The Natural killer (NK) cells are a subset of lymphocytes comprising around 10% of total lymphocytes in peripheral blood (1). Due to their role in the innate response, NK cells provide the “first line of defense” against infectious agents and tumor cells (2-5). The ability of NK cells in spontaneously lysing tumor cells and their effective anti-leukemic cytotoxic activity in vitro are suggestive of functions of these cells in “in vivo” anti-leukemia immune response (2,6,7).

The activity of NK cells is regulated by several receptor systems. Killer cell immunoglobulin-like receptors (KIR) on NK cells and their ligands, i.e. Human Leucocyte Antigen (HLA) class I molecules, play a critical role in this tight regulation (8). The KIR2DL1 binds HLA-Cw2/4/5/6/15 allotypes (HLA-C2 group) that carry a lysine residue at amino acid position 80. KIR2DL2 and 2DL3 recognize the remaining of the HLA-C allotypes (Cw1/3/7/8- HLA- C1 group) that carry an asparagine at the same position (9-12). KIR3DL1 binds to allotypes with Bw4 motif (HLA-Bw4), a serologically defined public epitope in  $\alpha$ 1 domain (residues 77-83) that presents on 40% of the HLA-B allotypes (13-15) and certain HLA-A molecules (HLA-A23, A24, A25, and A32) (16,17). The dimorphic position 80 among the HLA-B Bw4-containing allotypes (Bw4<sup>Isoleucine80</sup> and Bw4<sup>Threonine80</sup>) affects its interaction with KIR3DL1 subtypes, where HLA-B Bw4<sup>Ile80</sup> generally exhibits stronger inhibition through KIR3DL1 compared to HLA-B Bw4<sup>Thr80</sup> (18,19). The activating receptors KIR2DS1, 2DS2 and 3DS1 are thought to share HLA ligand binding specificities with their structurally related inhibitory counterparts (*KIR2DL1*, *2DL2/3* and *3DL1*, respectively) (20,21). The balance between the signals generated by these receptor-ligand pairs could lead to either triggering or blocking NK cell activity (22-24).

To date, 15 *KIR* genes and 2 pseudogenes have been described. Eight genes encode for the inhibitory KIR (iKIR) receptors (2DL1-3, 3DL1-3, 2DL5A/B), six genes encode for the activating KIR (aKIR) receptors (2DS1-5, 3DS1), one gene encodes for KIR2DL4 receptor with both inhibitory and activating functions, and two genes (*2DPI* and *3DPI*) are pseudogenes that do not encode a functional KIR receptor. Two basic *KIR* haplotypes have been defined on the basis of gene content, and are termed haplotypes A and B. While the B haplotype has many activating genes, the A haplotype has one, and in many occasions, this gene (*KIR2DS4*) is present in a deleted form which is not believed to be expressed at the cell surface. Therefore, individuals with two A haplotypes (AA genotype) may not have any activating *KIR* gene (25,26).

Several studies have shown lowered expression of HLA class I molecules in leukemia cells (27-29). Downregulation of HLA class I expression in leukemia cells relieves the inhibitory influence on NK cells, permitting NK cells to lyse these unhealthy target cells, a phenomenon first described as the ‘missing-self’ hypothesis (30). Furthermore, recent studies support a major role for NK cells in the graft-versus leukemia effect in allogeneic stem cell transplantation (31-33). Altogether, these data demonstrate that NK cells play a major role in the innate immune surveillance of leukemic cells (34).

As *KIR* at chromosome 19q13.4 and *HLA* at chromosome 6p21.3 display extensive polymorphism, the independent segregation of these gene loci produce variations in the number and type of *KIR+HLA* pairs and *aKIR* genes inherited in individuals (35). Consequently, the variable compound *KIR-HLA ligand* genotypes in a population may affect NK mediated innate immunity to control leukemia (34). In leukemia patients, failure in the clearance of leukemic cells could be caused by decreased activity of NK cells,

which may be the result of genetically determined *KIR-HLA ligand* gene content, leading to a dominance of inhibition over activation (36). This prompted us to investigate whether *KIR* and their known *HLA ligand* genes have predisposing roles in the occurrence of acute leukemia. In this study, we examined the protection/susceptibility to acute leukemia by analysing the frequencies and combinations of *KIR* and *HLA ligand* genes in patient groups and compared the results with a healthy control population.

## MATERIALS AND METHODS

**Patients and Controls.** The patient groups consisted of 78 unrelated Iranian individuals with acute myeloid leukemia (AML; n=40; 25 males and 15 females; range 11-46 years) and acute lymphoid leukemia (ALL; n=38; 28 males and 10 females; range 13-33 years). The institutional review board of the Bone Marrow Transplantation Center of Shariati Hospital (Tehran, Iran) approved the use of patient samples for this study. All samples were collected with the written consent of the patients or of their legal guardians. The control group consisted of 200 unrelated, healthy, Iranian individuals (100 males and 100 females; range 20-50 years) whose *KIR-HLA* genotypes were characterized elsewhere (37). DNA samples were prepared from peripheral blood leukocytes by the salting-out method (38) and their quality and quantity were determined by ultraviolet spectrophotometry.

**Combined *KIR-HLA Ligand* Genotyping.** DNA samples were tested for the presence or absence of 17 *KIR* genes (*2DL1*, *2DL2*, *2DL3*, *2DL4*, *2DL5A*, *2DL5B*, *2DS1*, *2DS2*, *2DS3*, *2DS4*, *2DS5*, *3DL1*, *3DL2*, *3DL3*, *3DS1*, *2DP1* and *3DP1*) and their three major *HLA class I* ligands (*C1*, *C2* and *Bw4*), in a combined genotyping assay as previously described (37,39). In addition, non-deleted (\*001) and deleted (\*003/004/006/007/009) versions of *KIR2DS4* gene were discriminated in all individuals. Only *2DS4\*001* allele was considered as a functional activating gene in the analyses. Each individual *KIR* genotype was determined to be AA when only the activating gene *KIR2DS4* was present. Any individual with only the deleted version of *KIR2DS4* was taken as having AA-zero (standing for zero *aKIR* gene) genotype. Other genotypes (AB or BB) which had additional activating genes besides *2DS4* were considered together as Bx (40).

We grouped *HLA-A*, *HLA-B* and *HLA-C* alleles according to known ligand specificity for the following major inhibitory and activating *KIR-HLA* combinations: (i) *KIR2DL2/L3* and *KIR2DS2* with *HLA-C1* alleles; (ii) *KIR2DL1* and *KIR2DS1* with *HLA-C2* alleles; and (iii) *KIR3DL1* and *KIR3DS1* with *HLA-B* and *HLA-A* allotypes that contained the Bw4 epitope (*HLA-Bw4* alleles). Moreover, we considered two subgroups (*Bw4<sup>11e80</sup>* and *Bw4<sup>Thr80</sup>*) in the analyses involving *HLA-B Bw4* alleles. *KIR3DL2* pairing with *HLA-A3/A11* private allotypes were not included in our study. We also determined the presence of six *aKIR* genes in different *iKIR+HLA* pairs.

**Statistical Analysis.** Carrier frequencies (CF) for *KIR* genes and *HLA ligands* were determined by direct counting. Additionally, distribution in the patient and control groups for *KIR* genotypes (AA, AA-zero, and Bx), inhibitory and activating *KIR+HLA* pairings, and genotype status regarding *iKIR+HLA / aKIR+HLA* and *iKIR+HLA / aKIR* (>, =, <) were defined. The significance of association was determined by  $\chi^2$  test with Yate's correction and Fisher's exact test when relevant. All presented p values are uncorrected and labeling with <sup>(\*)</sup> indicates values which remain significant (p<0.05) after correction. The odds ratio (OR) was calculated by the cross-product ratio. Exact confi-

dence interval (CI) of 95% was obtained. For assessing the consistency of genotype distribution with Hardy-Weinberg equilibrium,  $\chi^2$  test was used.

## RESULTS

### Frequencies of *KIR* Genes and Genotypes in the Controls and AML/ALL Patients.

The distribution of carrier frequencies for *KIR* genes in AML patients, ALL patients and control group is presented in Table 1. The frequency of *KIR* genes was almost the same in all groups. Only the frequencies of *KIR2DS3* and *KIR3DS1* were less common in AML patients. The frequency of *KIR2DS3* was significantly higher in controls than the AML group (38% vs. 12.5%, OR= 0.23, CI= 0.09-0.62, p=0.0018\*). Also, *KIR3DS1* was more frequent in control subjects compared to AML patients (44.5% vs. 27.5%, OR=0.47, CI=0.22-0.99, p=0.0465), although significance was lost after correction (Table 1).

**Table 1. Comparison of carrier frequencies of *KIR* genes, their *HLA* ligands, and *KIR* genotypes in the controls and AML/ALL patients.**

<i>KIR</i> Genes	Controls (n=200) % F	AML patients (n=40) % F	ALL patients (n=38) % F
<b>Inhibitory</b>			
<i>2DL1</i>	96.5	92.5	92.2
<i>2DL2/3</i>	100	100	100
<i>2DL2</i>	56.5	47.5	55.2
<i>2DL3</i>	86.5	85.0	81.7
<i>2DL4</i>	100	100	100
<i>2DL5</i>	61.5	50.0	52.5
<i>2DL5A</i>	40.0	45.0	39.5
<i>2DL5B</i>	35.0	25.0	28.9
<i>3DL1</i>	91.5	97.5	84.2
<i>3DL2</i>	100	100	100
<i>3DL3</i>	100	100	100
<b>Activating</b>			
<i>2DS1</i>	45.5	42.5	44.6
<i>2DS2</i>	57.5	52.5	55.2
<i>2DS3</i>	38.0	12.5 <sup>a</sup>	36.8
<i>2DS4</i>	91.5	97.5	84.2
<i>2DS4-full</i>	32.0	22.5	28.9
<i>2DS4-del</i>	82.5	82.5	76.3
<i>2DS5</i>	40.0	40.0	39.4
<i>3DS1</i>	44.5	27.5 <sup>b</sup>	39.4
<b>Pseudogene</b>			
<i>2DP1</i>	96.5	97.5	92.2
<i>3DP1</i>	100	100	100
<i>3DP1-full</i>	30.5	25.0	21.1
<i>3DP1-del</i>	97.0	97.5	94.7
<b>HLA ligands</b>			
<i>C1</i>	76.0	80.0	73.6
<i>C2</i>	72.0	67.5	86.9
<i>Bw4</i>	76.5	80.0	84.2
<i>B Bw4<sup>Thr80</sup></i>	10.0	12.5	7.90
<i>B Bw4<sup>Ile80</sup></i>	56.5	70.0	68.4
<i>A Bw4</i>	36.0	47.5	36.8
<b><i>KIR</i> genotypes</b>			
AA	8.5	10.0	13.2
AA-zero	19.0	25.0	18.5
Bx	72.5	65.0	68.3

F: Frequency; <sup>a</sup> Corrected Significant differences; <sup>b</sup> Significant difference without correction.

We identified a total of 19 different *KIR* genotypes (presence/absence of *KIR* genes) in leukemia patients vs. 26 genotypes in the control individuals (detailed genotype data is

not shown). The AA, AA-zero, and Bx (AB+BB) genotypes were counted in AML and ALL groups and compared to those obtained in the controls (Table 1). The AA-zero genotype was more frequent in AML patients than the control subjects (25% vs. 19%), whereas Bx genotypes had inverse distribution (65% vs. 72.5%). However, there was no significant difference in the frequency of *KIR* genotypes among study groups (Table 1).

**Frequencies of HLA Ligand Genes and Genotypes in the Controls and AML/ALL Patients.** The carrier frequencies of *HLA ligand* genes in the AML patients, ALL patients and controls are illustrated in Table 1. In this study, *HLA-B Bw4<sup>lle80</sup>* was more common in subjects with AML compared to controls (70% vs. 56.5%,  $p=0.1134$ ). In addition, *HLA-C2* was found more frequently in ALL group compared to control subjects (86.9% vs. 72%,  $p=0.0547$ ).

Six *KIR ligand* genotypes were identified in our study groups. The most common genotype, genotype 1 (presence of *C1*, *C2*, and *Bw4*), demonstrated an increased frequency in ALL patients compared to the controls (55.2% vs. 37.5%,  $p=0.0407$ ). This difference failed to reach a significant level after correction (Table 2).

**Table 2. The frequencies of HLA ligand genotypes in the controls and AML/ALL patients.**

<i>HLA ligands</i> genotype #	<i>HLA-C1</i>	<i>HLA-C2</i>	<i>HLA-Bw4</i>	% of con- trols (n=200)	% of AML patients (n=40)	% of ALL patients (n=38)
1				37.5	40	55.2 <sup>a</sup>
2				23	22.5	10.5
3				16	17.5	18.5
4				10.5	7.5	5.3
5				8	2.5	7.9
6				5	10	2.6

<sup>a</sup>Significant difference without correction.

Note: Gray rectangles indicate presence and white rectangles indicate absence of *HLA ligand* genes.

**Relationship between KIR and HLA Ligand Genes in the Controls and AML/ALL Patients.** Although 89% of the controls and 84.6% of the patients carried three *iKIR* genes (*2DL1*, *2DL2/3* and *3DL1*), their ability to trigger inhibitory responses depends on the availability of the cognate HLA class I ligands. To determine the distribution of functional *iKIR* receptor, we established the frequency of the three *iKIR+HLA* pairs i.e. *2DL1+HLA-C2*, *2DL2/3+HLA-C1* and *3DL1+HLA-Bw4* in the control and patient groups (Table 3). The *2DL1+HLA-C2* was more common in ALL patients compared to control subjects (81.6% vs. 69.5%,  $p=0.1309$ ). We also analyzed whether subgroups of *Bw4* ligand are involved in the susceptibility to acute leukemia. The *3DL1+HLA-Bw4<sup>lle80</sup>* and *3DL1+HLA-A Bw4* genotypes were found to increase in the AML patients compared to control group (67.5% vs 52.5%,  $p=0.0817$  and 45% vs 31%,  $p=0.0864$ , respectively) ( Table 3).

Furthermore, we hypothesized that the activating *KIR* genes might be more important in regulating the function of NK cells in acute leukemia. Thus, we explored the relationship between the presence of activating *KIR2DS1* in individuals expressing *HLA-C2*, *KIR2DS2* in individuals expressing *HLA-C1* and *KIR3DS1* in individuals expressing *HLA-Bw4*. The *KIR2DS2+HLA-C1* (35% vs. 44%,  $p=0.2948$ ) and *KIR3DS1+HLA-Bw4* (20% vs. 33%,  $p=0.1041$ ) were less common in subjects with AML compared to control

individuals. The other *aKIR+HLA* genotypes had nearly the same distribution in all groups (Table 3).

**Table 3. The frequencies of co-inheritance of inhibitory and activating *KIR* genes and their specific *HLA class I ligands* in the controls and AML/ALL patients.**

<i>KIR+HLA</i> combinations	Controls (n=200) % F	AML patients (n=40) % F	ALL patients (n=38) % F
<b>Inhibitory</b>			
<i>2DL2/3+CI</i>	76.0	80.0	73.7
<i>2DL1+C2</i>	69.5	62.5	81.6
<i>3DL1+Bw4</i>	71.0	77.5	68.4
<i>3DL1+B Bw4 T</i>	8.5	10.0	7.9
<i>3DL1+B Bw4 I</i>	52.5	67.5	52.6
<i>3DL1+A Bw4</i>	31.0	45.0	34.2
<b>Activating</b>			
<i>2DS2+CI</i>	44.0	35.0	47.4
<i>2DS1+C2</i>	31.0	30.0	34.2
<i>3DS1+Bw4</i>	33.0	20.0	26.3
<i>3DS1+B Bw4 T</i>	6.5	2.5	0.0
<i>3DS1+B Bw4 I</i>	23.0	23.0	23.7
<i>3DS1+A Bw4</i>	19.5	15.4	5.3

F: Frequency

**The Number and Type of *iKIR+HLA* and *aKIR+HLA* Combinations in Controls and AML/ALL Patients.** To determine the least number of *iKIR* receptor for cognate *HLA class I* molecule, we analysed the number and type of *iKIR+HLA* pairs inherited in the controls and patients. The frequency of *2DL2/3+CI* and *3DL1+Bw4* combinations (two inhibitory pairs) was lower in ALL patients compared to controls (10.5% vs. 23.5%,  $p=0.0740$ ) (Table 4). Whereas, ALL patients with all three inhibitory pairs were more frequent than the control group (39.5% vs. 31.5%,  $p=0.3371$ ). Furthermore, the number and type of inherited *aKIR+HLA* pairs in the controls and patients were determined. Nearly the frequency of three, two, and one *aKIR+HLA* pair(s) were the same in all groups (Table 4). However, the frequency of none of the *aKIR+HLA* pair(s) was higher in AML patients than control individuals (45% vs. 35.5%,  $p=0.2570$ ).

**Table 4. Combination of inhibitory and activating *KIR+HLA* pairs and their frequencies in the controls and AML/ALL patients.**

Number of pairs	<i>KIR+HLA</i>	Controls (n=200) % F	AML patients (n=40) % F	ALL patients (n=38) % F
<b>Inhibitory</b>				
Three	<i>2DL2/3+CI</i> , <i>2DL1+C2</i> and <i>3DL1+Bw4</i>	31.5	35.0	39.5
Two	<i>2DL2/3+CI</i> and <i>2DL1+C2</i>	14.5	7.5	15.8
	<i>2DL2/3+CI</i> and <i>3DL1+Bw4</i>	23.5	27.5	10.5
	<i>2DL1+C2</i> and <i>3DL1+Bw4</i>	15.5	15.0	18.4
	<i>2DL2/3+CI</i>	6.5	10.0	7.9
One	<i>2DL1+C2</i>	8.0	5.0	7.9
	<i>3DL1+Bw4</i>	0.5	-	-
<b>Activating</b>				
Three	<i>2DS2+CI</i> , <i>2DS1+C2</i> and <i>3DS1+Bw4</i>	10.0	2.5	10.5
Two	<i>2DS2+CI</i> and <i>2DS1+C2</i>	6.0	10.0	7.9
	<i>2DS2+CI</i> and <i>3DS1+Bw4</i>	6.5	2.5	5.3
	<i>2DS1+C2</i> and <i>3DS1+Bw4</i>	12.0	12.5	7.9
One	<i>2DS2+CI</i>	21.5	20.0	23.7
	<i>2DS1+C2</i>	3.0	5.0	7.9
	<i>3DS1+Bw4</i>	5.5	2.5	2.6
None	-	35.5	45.0	34.2

F: Frequency.

**Relationship Between *iKIR+HLA* / *aKIR+HLA* and *iKIR+HLA* / *aKIR* Status in the Controls and AML/ALL Patients.** To determine the correlation between the *iKIR+HLA* and *aKIR+HLA* pairs, we analyzed the combined frequencies of these combinations in the study groups (Table 5). In addition, the presence of six *aKIR* genes in the context of different *iKIR+HLA* pairs was also assessed (Table 5). The Analysis of combined frequencies for *iKIR+HLA* and *aKIR+HLA* pairs revealed that the majority of controls and patients had *iKIR+HLA*>*aKIR+HLA* status. However, this kind of compound genotype had higher frequency in AML patients than controls (75% vs. 69%,  $p=0.4523$ ). Moreover, the frequency of genotypes with greater number of *iKIR+HLA* pair(s) than *aKIR* genes (*iKIR+HLA*>*aKIR*) was higher in subjects with AML compared to control individuals (45% vs. 35%,  $p=0.2314$ ). No statistically significant dissimilarity in the frequency of different compound *KIR-HLA* genotypes between patients and controls was found (Table 5).

**Table 5. The combined frequencies of different compound *KIR-HLA* genotypes in the controls and AML/ALL patients.**

<i>KIR-HLA</i> genotypes	Controls (n=200)	AML patients (n=40)	ALL patients (n=38)
	% F	% F	% F
<i>iKIR+HLA</i> > <i>aKIR+HLA</i>	69.0	75.0	63.2
<i>iKIR+HLA</i> = <i>aKIR+HLA</i>	25.0	22.5	23.7
<i>iKIR+HLA</i> < <i>aKIR+HLA</i>	6.0	2.5	13.1
<i>iKIR+HLA</i> > <i>aKIR</i>	35.0	45.0	47.4
<i>iKIR+HLA</i> = <i>aKIR</i>	20.0	20.0	5.2
<i>iKIR+HLA</i> < <i>aKIR</i>	45.0	35.0	47.4

F: Frequency

## DISCUSSION

NK cells participate in the innate immunity against tumor or virus-infected cells with abnormal expression of HLA class I molecules. It has been proposed that the expression of HLA class I molecules is down regulated in leukemic cells (29, 41-43). Apparently, the NK cells of patients with leukemia are not able to destroy these leukemic cells and may let the malignant cells to escape from innate immune control. This failure may be due to the fact that NK cells are a fraction of the malignant clone and thus might have a decreased activity (41). Alternatively, these patients may show a compound *KIR-HLA* genotype unable to destroy leukemic cells (34).

A previous study has reported a significant increase in *KIR2DL2* and *KIR2DS2* in Belgium Caucasian patients of acute and chronic myeloid and lymphoid leukemia (34). Although the numbers were small in some of the groups, this increase was obvious in all patients. A recent study has shown lowered frequencies, although not significant, of *KIR2DL2* and *KIR2DS2* in chronic myeloid leukemia (CML) patients and to a lesser extent in AML patients compared to controls (36). These results were in contrast to those of the present study. In this study, frequencies of *KIR2DL2* and *KIR2DS2* were the same in all groups. Conversely, the frequency of *KIR2DS3* was significantly decreased in AML group (Table 1). In addition, an association ( $p=0.0465$ ), not significant after correction, was observed for *KIR3DS1* in AML patients compared to the controls. The reason for such opposing results remains to be investigated.

In the study of Middleton et al. (36), when AA genotypes were analyzed to determine those with no activating *KIR* gene bar *KIR2DL4* (AA-zero), 18.52% of AML and 25%

of CML patients had this AA genotype compared to 12.5% of the controls. Likewise, the frequency of AA-zero genotype was non-significantly higher in our subjects with AML compared to the controls (25% vs. 19%) (Table 1). On the other hand, Bx genotypes were more common in the controls than in AML patients (72.5% vs. 65%). The above data may imply that the NK cells of a substantial number of AML patients are under an extra inhibitory condition caused by reduction of *KIR* B haplotypes and activating *KIR* genes.

It has been confirmed that *HLA* genes are involved in enhancing genetic susceptibility of leukemia. In addition, function of *KIR* on effector cells is highly dependent on the *HLA* class I molecules expressed on targets. We have shown a non-significant increase of *HLA-B Bw4<sup>Ile80</sup>* in the AML patients and a similar increase of *HLA-C2* and *HLA ligand* genotype no. 1 in the ALL group (Table 1 and 2). The presence of more *HLA* ligands could possibly lead to greater inhibition of NK cell activity via inhibitory *KIR* ligation.

As the regulation of NK cell response depends mainly on cognate recognition between products of *KIR* and *HLA* genes carried by an individual, it is proposed that the *KIR*-*HLA* relationship may be important in a disease such as leukemia. In this regard, we lay particular emphasis on compound *KIR*-*HLA* genotype to determine its relative contribution to AML/ALL susceptibility. In a study performed by Verheyden et al., (44) an increased frequency of *KIR2DL2* and *KIR2DS2* in combination with their ligand group (*HLA-C1*) has been reported in patients with myeloid leukemia (52.1% vs 31.7% in controls). In our study, the increase of *KIR3DL1+HLA-B Bw4<sup>Ile80</sup>* and *KIR3DL1+HLA-A Bw4* in AML and *KIR2DL1+HLA-C2* in ALL patients, although statistically not significant, may be effective in the enhancement of inhibition. Furthermore, decreased frequency of *KIR2DS2+HLA-C1* and *KIR3DS1+HLA-Bw4* in AML group may turn the NK balance to an added inhibitory situation (Table 3).

NK cells, in contrast to T and B cells, employ a multiple receptor recognition strategy, whereby each NK cell can be triggered by various receptors separately or in combination, depending on the ligands presented by the target cell in a given encounter. If a presumed NK cell utilizes both inhibitory and activating receptors to recognize the target, the balance between these counter signals ensures the action of that NK cell. In this study, analysis of combined frequencies for *iKIR+HLA* against *aKIR+HLA* pairs revealed that the majority of the study groups had *iKIR+HLA > aKIR+HLA* status, although this kind of compound genotype had higher frequency in AML patients than controls (75% vs. 69%,  $p = 0.4523$ ). On the other hand, the frequency of genotypes with greater number of *iKIR+HLA* pair(s) than *aKIR* genes (*iKIR+HLA > aKIR*) was higher in subjects with AML compared to the control individuals (45% vs. 35%,  $p = 0.2314$ ). Once again, these findings may represent a state of NK hyporesponsiveness through *KIR*-*HLA* and *aKIR* pathways in these patients.

In conclusion, the only significant difference found in the present study is related to the lower frequency of *KIR2DS3* in AML patients than the control group. In addition, other non-significant differences in this patient group including decreased occurrence of *KIR3DS1*, deviated combined frequency of *KIR+HLA* combinations, and high rate of *iKIR+HLA > aKIR+HLA* and *iKIR+HLA > aKIR* compound genotypes had trends toward less activating and more inhibitory conditions. Interestingly, this situation was not observed in ALL patients and the inhibition enhancement through increases of *HLA ligands* and inhibitory combinations was the main feature in this group. It is noteworthy that in the use of *HLA* haplotype-mismatched donors, a significant graft vs. leukemia



effect was observed in AML but not in ALL patients (45). Moreover, a recent report has shown a significant improvement in graft survival at 3 years in AML patients in non-T-cell-depleted unrelated donor stem cell transplantation when the donor had at least one B haplotype (i.e. presence of *KIR2DL5*, *2DS1*, *2DS2*, *2DS3*, *2DS5*, or *3DS1*) (46). Thus, a genetic imbalance between activating and inhibitory *KIR* genes and *KIR+HLA* combinations might have an influence on the susceptibility to acute leukemia by up-regulation of inhibition or by the loss of activation or a mixture of both, to favor the escape of malignant cells from NK cell immunity. Evaluating the role of other receptor-ligand pairs involved in NK regulation may, however, better clarify further aspects of leukemia escape.

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