Killer cell immunoglobulin-like receptors in Thai patients with leukemia and diffuse large B-cell lymphoma

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Abstract

Natural killer (NK) cells are key components of the innate immune system that have been implicated in the immune response against tumor cells. Killer cell immunoglobulin-like receptors (KIR) regulate NK cell activity by interaction with specific human leukocyte antigen (HLA) class I. In this study, KIR gene polymorphisms and their HLA ligands were investigated in Thai patients with chronic myelogenous leukemia (CML) (n = 60), acute myelogenous leukemia (AML) (n = 60), acute lymphoblastic leukemia (ALL) (n = 55), and diffuse large B-cell lymphoma (DLBCL) (n = 60) compared with 150 healthy controls. The frequency of KIR3DL1 with HLA-Bw4 was significantly lower in DLBCL patients than in controls (P = 0.0006, Pc = 0.02), whereas no significant differences were seen in KIR gene frequencies and their ligands between leukemia patients and controls. This study suggests a role of inhibitory KIR with its ligand in the protection against DLBCL.

1. Introduction

Leukemia and lymphoma are common hematological malignancies. In lymphoma, diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL). The immune surveillance of tumor cells depends to a large extent on T cell or natural killer cell (NK) reactivity. HLA class I molecules are important in the interaction with T- and NK- specific receptors. Previous studies have shown that malignant cells can escape from elimination by cytotoxic T cells by a reduction or loss of some HLA class I molecules, which in turn exposes them to the attack from NK cells [1,2].

Natural killer cells are the key components of the innate immune response and have the ability to kill tumor and virus-infected cells [3,4]. The killer cell immunoglobulin-like receptors (KIR) are cell surface receptors of the immunoglobulin superfamily that are expressed on NK cell and subsets of activated or memory T lymphocytes in human. KIR receptors are divided into functional inhibitor groups that prevent target cell lysis and activators that incite cell lysis. KIR with long cytoplasmic tail (L) transmit inhibitory signals after binding their cognate ligand via immuno-tyrosine inhibitory motifs (ITIM), while those with short cytoplasmic tails (S) send activating signals via adapter molecules DAP12.

KIR are encoded by a family of highly polymorphic genes clustered in the leukocyte receptor complex on chromosome 19q13.4. KIR exhibits extensive genotypic diversity due to gene number and component [5]. Several KIR receptors use HLA molecules as their ligand. The ligand for KIR2DL1 and 2DL3 is the C2 group (HLA-Cw alleles with lysine at position 80). The ligand for KIR2DL2 and 2DL3 and 2DS2 is the C1 group (HLA-Cw alleles with asparagine at position 80). The ligand for KIR3DL1 is Bw4 [5]. HLA-Bw4 can be divided into two groups on the basis of whether isoleucine or threonine is present at position 80. Receptor binding and lysis inhibition data suggest that Bw4 80Ile may be more effective ligands for KIR3DL1 than Bw4 80Thr [6]. Epidemiological studies implicate HLA-Bw4 ligand for KIR3DS1 [7]. The effects of KIRs on host immune responses are mediated by specific interaction of these receptors and HLA class I ligands, which result in either activating or inhibition of NK cell cytotoxicity. In hematological malignancies, the associations of KIR genes and HLA ligands were reported in leukemia, however the results are controversial [17–23] and to our knowledge, the association of KIR genes with NHL has not been reported. The present study was undertaken to investigate the association of KIR genes and their ligands with leukemia and DLBCL patients in Thai population.
2. Materials and methods

2.1. Study subjects

The subjects studied consisted of four groups of Thai patients diagnosed at hematology clinic, Department of Medicine, Siriraj Hospital, Bangkok. The first group included 60 patients with chronic myelogenous leukemia (CML) (30 males and 30 females, mean age 42 years, range 19–79 years). The second group included 60 patients with acute myelogenous leukemia (AML) (30 males and 30 females, mean age 42 years, range 19–79 years). The third group included 55 patients with acute lymphoblastic leukemia (ALL), (31 males and 24 females, mean age 25 years, range 11–65 years). The fourth group included 60 patients with DLBCL (30 males and 30 females, mean age 49 years, range 20–76 years). All the patients were HIV negative. A control group included 150 healthy Thai individuals from blood donors at Siriraj Hospital. All subjects were interviewed about their ancestry and were from the central Thai ethnic background. Written informed consent was provided by all the subjects and the study was approved by the central Thai ethic background. 

2.2. HLA and KIR genotyping

HLA-C and Bw4 typing were done by PCR-SSP (polymerase chain reaction with sequence specific primer) [8]. Validation of the method was done by using Micro-SSP (one lambda, Canoga Park, CA) in randomly selected samples and external quality program. Typing Individuals were categorized as HLA-C1 and HLA-C2 on the basis of their genotyping data. HLA-Bw4 epitope typing was done by PCR-SSO [9]. HLA-Bw4 was only considered on the HLA-B locus.

The presence or absence of KIR genes was detected using PCR-SSP (polymerase chain reaction with sequence specific primer) [10,11]. Typing method was applied in previous studies and in external quality control program [12,13]. In brief, the reactions of 10 μl were set up to include 0.1 μg test DNA, buffer IV, 0.2 mM dNTP, 1.08 mM magnesium chloride, 0.3 U Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany) and 0.5 μM specific primer mix (except for 3DL1, 2DS4 which were at final concentration of 1 μM). Internal controls (5′-CAGTGCCTTCCCAACCATTCCCTTA-3′, 5′-ATTCACCTACCGGATTTCTGTTGTGTTTC-3′) specific for a 485 basepair human growth hormone fragment were included at 0.067 μl in each reaction. All amplifications were performed in duplicate in Perkin Elmer 9700 (PE Biosystem, California, USA) under thermal cycling conditions as follows 5 min denaturing step at 94 °C, 10 cycles of 94 °C 10 s, 65 °C 60 s, then 20 cycles of 94 °C 10 s, 61 °C 50 s, 72 °C 30 s. The products were photographed from standard 1% agarose electrophoresis gels containing ethidium bromide.

2.3. Statistical analysis

Carrier frequencies for KIR genes, and HLA ligands were determined by direct counting. In addition, KIR genotypes and KIR genes with their ligands were determined. The differences in frequencies between patients and controls were done by Pearson's Chi-square test. Fisher exact tests were performed when relevant. The odds ratios (OR) and 95%CI were also calculated. P values less than 0.05 were considered to be significant. The Bonferroni correction for multiple comparisons was also applied. Statistical data were calculated by using the SPSS version13.0 program. A power calculations were also performed using the PS software program and 80% power was used to calculate sample size.

3. Results

The distribution of KIR genes and genotypes in patients and controls is shown in Table 1. Framework genes KIR2DL4, KIR3DL2, KIR3DL3 and KIR3DP1 were present in all samples. KIR genes with high frequency or more than 90% are KIR2DL1, KIR2DL3, KIR3DL1, KIR2DS4. There were no significant differences in the frequencies of KIR genes between each group of the patients and controls. In addition, the frequencies of KIR AA, Bx genotypes were not significantly different between each group of the patients and controls. KIR AA was determined when only activating gene 2DS4 was present, while the remaining were considered as Bx. [14] The frequencies of HLA-Bw4 and HLA-C ligands are shown in Table 2. The frequency of HLA-C2 was significantly lower (23.3% vs. 38%, P = 0.04, OR = 0.5), whereas the frequency of HLA-C1 was significantly higher (76.7% vs. 62%, P = 0.003, OR = 2.01) in AML patients than in controls. The frequency of Bw4 was significantly lower in DLBCL patients than in the controls (43.3% vs. 66%, P = 0.003, OR = 0.39). However, the statistical differences for these ligands were lost after P correction. When Bw4 epitopes (Bw4 80T and Bw4 80I) were analyzed, only the frequency of Bw4 80T was significantly lower in DLBCL patients than in controls (16.7% vs. 33.3%, P = 0.02, OR = 0.4). There were no significant differences in the frequencies of HLA-C1, C2 and Bw4 in CML and ALL patients compared with controls. The presence of KIR genes with their HLA ligands is shown in Table 3. The frequency of KIR2DL1 with C2 was significantly lower in AML patients than in controls (21.7% vs. 36%, P = 0.04, OR = 0.49), however this was not significant after P.
NK cells are thought to be important in eliminating tumor cells especially leukemia, lymphoma and metastatic tumor cells [15,16]. The associations KIR genes and leukemia were reported in different groups, but the results are controversial (Table 4). In addition, most of the studies were performed in Caucasians. In myeloid malignancies, a study on the Belgium Caucasian population found a significant increase in KIR2DL2 and 2DS2 with HLA-C1 in myeloid leukemia patients [17], however a study on the Turkish population found a significant decrease in KIR2DL2 and KIR2DS2 with HLA-C1 in CML patients [18]. This group also found a significant increase in KIR2DL2 + C2 with protection against the disease [23].

In our study, we could not confirm association of KIR gene with CML, AML and ALL. Although a decrease in KIR2DL1 + HLA-C2 was found in AML, the significance is weak and the difference was not significant after P correction. The difference in the findings of this study from those of previous studies in leukemia can be attributed to several factors. First, the frequencies of KIR genes or ligands might affect statistical significance. Further studies in larger sample size for each group of leukemia are needed. Interestingly, our study found a protective role of KIR3DL1 + HLA-Bw4 in DLBCL. In addition, Bw4 80T was significantly reduced in DLBCL patients than in controls (36.7% vs. 62.7%, P = 0.0006, OR = 0.002, OR = 0.34). There was no significant difference in the interaction of other KIR and HLAs between patients and controls.

4. Discussion

NK cells are thought to be important in eliminating tumor cells especially leukemia, lymphoma and metastatic tumor cells [15,16]. The associations KIR genes and leukemia were reported in different groups, but the results are controversial (Table 4). In addition, most of the studies were performed in Caucasians. In myeloid malignancies, a study on the Belgium Caucasian population found a significant increase in KIR2DL2 and 2DS2 with HLA-C1 in myeloid leukemia patients [17], however a study on the Turkish population found a significant decrease in KIR2DL2 and KIR2DS2 with HLA-C1 in CML patients [18]. This group also found a significant increase in HLA-Bw4 in CML patients. In Asian population, a study on the Chinese population found significant association of KIR2DS4 with susceptibility to CML [19], however this study did not differentiate full length and deletion allele of KIR2DS4. In contrast, a study on the Polish population found protective effect of full length KIR2DS4 allele with CML. In lymphoid malignancies, most of the studies found no association of KIR genes with ALL [17,18,21]. However, a study on the Canadian Caucasian population found significant association of activating KIR with reduced risk for childhood ALL [22]. In CLL, a study on the Belgium Caucasian population found association of KIR3DL1 + HLA-Bw4 with susceptibility to the disease [17], in contrast, a study on the Polish population with much larger sample size found association of KIR3DL1 + HLA-Bw4 with protection against the disease [23].

In our study, we could not confirm association of KIR gene with CML, AML and ALL. Although a decrease in KIR2DL1 + HLA-C2 was found in AML, the significance is weak and the difference was not significant after P correction. The difference in the findings of this study from those of previous studies in leukemia can be attributed to several factors. First, the frequencies of KIR genes or ligands might affect statistical significance. Further studies in larger sample size for each group of leukemia are needed. Interestingly, our study found a protective role of KIR3DL1 + HLA-Bw4 in DLBCL. In addition, Bw4 80T was significantly reduced in DLBCL. The result was similar to the association found in CLL [23]. Although there is evidence that Bw4 80I showed stronger interaction with KIR receptor than Bw4 80T [6], it is possible that Bw4 80T may also be important. However, in this study, HLA-A- Bw4 was not included. Although HLA-B epitopes are regarded as important KIR3DL1 ligands, it was suggested that HLA-A might be important correction. The frequency of KIR3DL1 with HLA-Bw4 was significantly lower in DLBCL patients than in controls (36.7% vs. 62.7%, P = 0.0006, OR = 0.02, OR = 0.34). There was no significant difference in the interaction of other KIR and HLAs between patients and controls.

Table 2

<table>
<thead>
<tr>
<th>KIR ligands</th>
<th>CML N = 60, n (%)</th>
<th>AML N = 60, n (%)</th>
<th>ALL N = 55, n (%)</th>
<th>DLBCL N = 60, n (%)</th>
<th>Control N = 150, n (%)</th>
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<tbody>
<tr>
<td>C1</td>
<td>55(91.7)</td>
<td>58(96.7)</td>
<td>54(98.2)</td>
<td>56(93.3)</td>
<td>141(94)</td>
</tr>
<tr>
<td>C2</td>
<td>23(38.3)</td>
<td>14(23.3)</td>
<td>17(30.9)</td>
<td>20(33.3)</td>
<td>57(38)</td>
</tr>
<tr>
<td>C1/C1</td>
<td>37(61.7)</td>
<td>46(76.7)</td>
<td>38(69)</td>
<td>40(66.7)</td>
<td>93(62)</td>
</tr>
<tr>
<td>C1/C2</td>
<td>18(30)</td>
<td>12(20)</td>
<td>16(29.1)</td>
<td>16(26.7)</td>
<td>48(32)</td>
</tr>
<tr>
<td>C2/C2</td>
<td>5(8.3)</td>
<td>2(3.3)</td>
<td>1(1.8)</td>
<td>6(7.7)</td>
<td>9(6)</td>
</tr>
<tr>
<td>Bw4</td>
<td>33(55)</td>
<td>34(56.7)</td>
<td>33(60)</td>
<td>26(43.3)</td>
<td>99(66)</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>KIR genes and their ligands</th>
<th>CML N = 60, n (%)</th>
<th>AML N = 60, n (%)</th>
<th>ALL N = 55, n (%)</th>
<th>DLBCL N = 60, n (%)</th>
<th>Controls N = 150, n (%)</th>
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</thead>
<tbody>
<tr>
<td>2DL1 + C2</td>
<td>21(35)</td>
<td>13(21.7)</td>
<td>15(27.3)</td>
<td>18(30)</td>
<td>54(36)</td>
</tr>
<tr>
<td>2DL2 + C1</td>
<td>23(38.3)</td>
<td>19(31.7)</td>
<td>22(40)</td>
<td>18(30)</td>
<td>56(37.3)</td>
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<tr>
<td>2DL2 + C2</td>
<td>52(86.7)</td>
<td>54(90)</td>
<td>52(94.5)</td>
<td>53(88.3)</td>
<td>132(88)</td>
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<tr>
<td>3DL1 + Bw4</td>
<td>32(53.3)</td>
<td>34(56.7)</td>
<td>32(58.2)</td>
<td>22(36.7)</td>
<td>94(62.7)</td>
</tr>
<tr>
<td>2DS1 + C2</td>
<td>14(23.3)</td>
<td>5(8.3)</td>
<td>7(12.7)</td>
<td>7(11.7)</td>
<td>21(14)</td>
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<tr>
<td>2DS2 + C1</td>
<td>23(38.3)</td>
<td>19(31.7)</td>
<td>21(38.2)</td>
<td>19(31.7)</td>
<td>55(36.7)</td>
</tr>
<tr>
<td>3DS1 + Bw4</td>
<td>12(20)</td>
<td>14(23.3)</td>
<td>9(16.4)</td>
<td>10(16.7)</td>
<td>36(24)</td>
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</table>

Table 4

<table>
<thead>
<tr>
<th>Population</th>
<th>Predisposing KIR genes and ligands</th>
<th>Protective KIR genes and ligands</th>
<th>References</th>
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<tbody>
<tr>
<td>Belgium</td>
<td>KIR2DL2/2DS2 + C1 (myeloid leukemia)</td>
<td>KIR2DL2/2DS2 + C1(CML)</td>
<td>[17]</td>
</tr>
<tr>
<td>Turkey</td>
<td>Bw4(CML)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>China(Han)</td>
<td>KIR2DS4(CML)</td>
<td></td>
<td>[19]</td>
</tr>
<tr>
<td>Poland</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>Full length KIR2DS4(CML)</td>
<td>Activating KIR (childhood ALL)</td>
<td>[20]</td>
</tr>
<tr>
<td>Belgium</td>
<td>KIR3DL1 + Bw4 (CLL)</td>
<td></td>
<td>[17]</td>
</tr>
<tr>
<td>Poland</td>
<td>KIR3DL1 + Bw4 (CLL)</td>
<td></td>
<td>[23]</td>
</tr>
</tbody>
</table>

References
for NK cell function [24]. Previous study has shown that the binding of KIR3DL1 to Bw4 ligand depends upon the bound peptide as well as HLA and KIR3DL1 polymorphism [25]. Further study on the role of KIR3DL1 subtypes and the interactions with HLA-A and HLA-B should be performed.

Both DLL and DLBCL are lymphoid malignancies which are subsets of NHL. In Thais, DLL is very rare compared to Caucasians, whereas DLBCL is the most common type of NHL. HLA-Bw4 is ligand for both KIR3DL1 and KIR3DS1. We also observed a decrease in KIR3DS1 + HLA-Bw4, but the difference was not significant. This might be from a low frequency of KIR3DS1 in the controls. KIR3DS1 was found to be protective against Hodgkin’s lymphoma [26]. In contrast, this gene has been associated with increase risk in HPV induced cervical cancer [27] and EBV-associated nasopharyngeal carcinoma [28]. Although the susceptibility effects of inhibitory KIR-HLA on some cancers were reported [29,30], a protective role of inhibitory KIR-HLA was observed in DLBCL in this study and in cervical neoplasia [27]. It is known that etiologic cause of NHL and cervical neoplasia are associated with infectious agent [31]. Therefore, KIR genes might be important in the pathogenesis of virus induced malignancies, however, the role of inhibitory or activating KIR are still not clear. In summary, KIR3DL1-HLA-Bw4 was found to be associated with the protection against DLBCL, however the association should be confirmed in other populations. There were no associations of KIR genes and ligands with CML, AML and ALL in Thai patients.

Acknowledgments

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References

[6] Celia M, Longo A, Ferrara GB, Strominger JL, Colonna M. NK-specific natural killer cells are selectively inhibited by Bw4-positive HLA alleles with isoleucine