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KIR2DL5, a Novel Killer-Cell Receptor with a D0-D2 Configuration of Ig-Like Domains1,2

Carlos Vilches,3 Raja Rajalingam,* Markus Uhrberg,† Clair M. Gardiner,* Neil T. Young,* and Peter Parham4,*

Four novel killer-cell Ig-like receptor (KIR) genes were discovered by analysis of genomic DNA from a human donor. One gene, KIR2DL5, is expressed by subpopulations of NK cells and T cells, whereas expression of the other three genes could not be detected. KIR2DL5 has two extracellular Ig-like domains of the D0 and D2 type, a structural configuration that was previously unique to KIR2DL4. Although having a similar structure overall, the KIR2DL4 and KIR2DL5 receptors have distinctive amino acid sequences in the ligand-binding extracellular domains and differ in the transmembrane and cytoplasmic motifs that determine signal transduction. Whereas the KIR2DL4 gene is present on all KIR haplotypes and is expressed by all human NK cells, the KIR2DL5 gene is restricted to the “B” subset of KIR haplotypes and is clonally expressed by NK cells within an individual. Chimpanzee genes for KIR2DL4 and KIR2DL5 have been defined and are very similar in sequence to their human orthologs. The donor in whom KIR2DL5 was first detected bears two variants of it that differ by five nucleotide substitutions in the coding region. Although the substitutions are not predicted to affect gene expression, transcription of only one of the two KIR2DL5 variants could be detected. The Journal of Immunology, 2000, 164: 5797–5804.

Killer-cell Ig-like receptors (KIR),5 also known as killer-cell inhibitory receptors, are glycoproteins of the Ig superfamily expressed on the surfaces of human NK cells and a subset of T lymphocytes (1–5). They comprise 12 protein groups, possibly encoded by different genes (1–3, 6–10). KIR can be detected.

Although the substitutions are not predicted to affect gene expression, transcription of only one of the two KIR2DL5 variants could be detected. The Journal of Immunology, 2000, 164: 5797–5804.

The extracellular regions of KIR consist either of two (KIR2D) or three (KIR3D) Ig-like domains: the three domains of KIR3D are named D0 (membrane-distal), D1 (middle), and D2 (membrane-proximal). Two subgroups of KIR2D can be distinguished according to which pair of extracellular domains they have (see Fig. 1). KIR2D of type I have extracellular domains corresponding to the D1 and D2 domains of KIR3D and lack a D0 domain. In contrast, KIR2D of type II have extracellular domains corresponding to D0 and D2 of KIR3D and lack the D1 domain. In humans the majority of KIR2D are of type I, whereas type II has only been represented by KIR2DL4 (8).

The cytoplasmic tails of KIR are classified into short and long forms. The difference arises from variation in the position of the stop codon, which is caused by single nucleotide replacements or frame-shifts associated with insertion/deletions of 1–4 nucleotides in the exons encoding the transmembrane or cytoplasmic regions. Additional variability among KIR is conferred by allelic polymorphism (11, 12) and by alternative splicing of RNA (13). Finally, there is also variation within the human population in the number and combination of KIR genes an individual inherits (14).

Much current knowledge of KIR diversity and structure derives from studies on mRNA and less from investigation of the structure and organization of KIR genes (15, 16). Our previous in-depth analysis of two donors revealed that different KIR can have diverse frequencies of expression in NK cell clones (17), which makes it possible that KIR expressed by small numbers of NK cells could have been overlooked in analysis of cDNA. To gain better knowledge of the gene structure of KIR and to search for possible new KIR genes, we studied genomic DNA by PCR amplification with oligonucleotide primers recognizing conserved regions of known human KIR genes. With this approach, we obtained evidence for the existence of four previously unknown KIR genes. In this report, we characterize exons 2–5 of these genes6 and show that one of them, KIR2DL5, is expressed at the mRNA level. The structures, expression and population distribution of KIR2DL5 were then studied further.
KIR2DL5, A NOVEL KILLER-CELL RECEPTOR WITH D0-D2 DOMAINS

Materials and Methods

Genomic DNA amplification of exons 2–5 of four new KIR genes

Genomic DNA was obtained from a B-lymphoblastoid cell line established from PBMC of a healthy adult of Caucasian origin (donor NV) (17). Overlapping fragments spanning each pair of adjacent exons from exon 2 to exon 5 were generated for each new gene by PCR amplification with primers designed to recognize conserved or gene-specific sequences of human KIR (Table I). One hundred nanograms of DNA was amplified in 20 μl of PCR buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 0.001% gelatin, and 50 μM of each dNTP) containing 0.5 U of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA) and 10 pmol of each primer. The PCR profile was 2 min of denaturation at 96°C; 10 cycles of 10 s at 94°C, 20 s at 58°C, and 2 min at 72°C; and 20 cycles of 10 s at 94°C, 20 s at 58°C, and 2 min at 72°C.

Amplification of KIR2DL5 from cDNA

Total RNA was obtained from 2 × 10⁷ PBMC of donor NV with the RNeasy miniprep kit (Qiagen, Chatsworth, CA) and was used to perform rapid amplification of cDNA ends (RACE). First-strand cDNA was synthesized with Superscript II retrotranscriptase (Life Technologies, Rockville, MD) and either 3′-CDS primer (for 3′-RACE experiments) or 5′-CDS plus SMART II oligonucleotide primers (for 5′-RACE), according to the recommendations of the manufacturer (SMART RACE; Clontech Laboratories, Palo Alto, CA). For 3′-RACE experiments (19), 5 pmol of a forward primer specific for exon 3 of KIR2DL5 (LF196, 5′-TGTGGTGCCTCGAGGAGGACAT-3′) was combined with LRg1769. In all cases, 2.5 μl of Advantage 2 PCR buffer containing 1 μl of Advantage 2 polymerase mix (Clontech), PCR conditions were: initial denaturation for 2 min at 96°C; and then 38 cycles of 5 s at 94°C, 15 s at 68°C, and 3 min at 72°C. As a positive control of cDNA quality, each clone was also tested with primers for β-actin (17) and, as a negative control, cDNA from feeder cells was assayed for KIR2DL5 expression using the same PCR conditions.

PCR typing of the new KIR genes

Genomic DNAs isolated from the peripheral blood of 34 unrelated individuals from Negroid, Caucasian, and Mongoloid ethnic groups were tested by PCR for the presence of the four new KIR genes described in this work. In addition, 108 DNA samples from the Duesseldorf Bone Marrow Donor Registry were tested for the presence of KIR2DL5; donors in this panel were of Caucasian origin (70% German, 10% Turkish, 10% Italian, and 10% Hispanic). The primers used for typing each gene were as follows: F153 and Rt669 for KIR44; F153 and Rt667 for KIR48; Fd674 (5′-TCT GTTACTCTACCTCCCCA-3′; forward, exon 4) and R939 for KIR15; and F196 (5′-TGCTCGAGGGGAAGCAT-3′; forward, exon 3) and Ra964 for KIR2DL5 (Table I). PCR was performed in the same conditions that were used for the genomic cloning of these genes.

Sequence analysis

Alignment of KIR sequences was performed using the Wisconsin Package Version 10.0 (Genetics Computer Group, Madison, WI). A phylogenetic tree was constructed by a neighbor-joining method (20) using the PAUP 4.0b2a software package (David L. Swofford, University of Illinois, Urbana, IL). Confidence in the tree was assessed from 500 replicates by the bootstrap method (21).

Results

Identification of four novel KIR genes, of which one is expressed in PBMC

To establish the organization of exons coding for the extracellular domains of KIR and to search for possible new KIR genes, we performed a series of PCR amplifications from genomic DNA of a donor (NV) whose KIR had been previously studied by cDNA sequencing (17). Several combinations of primers recognizing either conserved or specific regions of KIR (Table I) were used to amplify and characterize expressed KIR in donor NV. This donor had been shown to express most of the known human KIR families (17), all of which we have identified and characterized in his genomic DNA (not shown). In addition, some clones carrying new KIR sequences were obtained in this study. These genomic DNA sequences differed from all published human KIR cDNAs and seemed to correspond to four new genes and/or pseudogenes, which were named KIR15, KIR44, KIR48 and, following the nomenclature used for KIR proteins (6, 14), KIR2DL5 (see below).

The new genes were first identified either in DNA segments spanning exons 2–3 (KIR2DL5 and KIR15), exons 3–4 (KIR15), or exons 4–5 (KIR44 and KIR48), as shown in Table I. To extend the sequence information on these new genes, we performed an “exon walking” approach (Table I) that generated overlapping fragments and provided the nucleotide sequence of exons 2–5 for all four new genes (Fig. 2). With the exception of KIR15, the exons sequenced from the new KIR genes bear no premature stop codons and conserve the reading frame seen in other KIR, and their putative exon/intron boundaries conform to the GT/AG rule.

Polymorphism was observed for three of the four new genes: KIR2DL5, KIR44, and KIR48. Two variants of each of these genes were found in donor NV, differing by 5, 4, and 1 nucleotide replacements, respectively, in exons 2–5 (Fig. 2). These changes were confirmed in PCR products obtained from different experiments and most likely reflect allelic polymorphism. The two variants of KIR44, KIR44a, and KIR44b are closely related to the nonexpressed KIRCI sequence recently reported by Torkar et al. (18), differing from it by 1 and 5 nucleotide substitutions, respectively. Thus KIR44a, KIR44b, and KIRCI probably constitute alleles of the same gene.
To determine whether the new genes are transcribed, the presence of their mRNAs was assessed in total RNA isolated from PBMC of donor NV. First-strand cDNA was synthesized and used as a template for PCR in which several combinations of specific primers were assayed for each of the new KIR (not shown). Only in the case of KIR2DL5 was an amplification product obtained, suggesting that the other three KIR genes are not expressed in PBMC. For KIR44, this apparent lack of expression agrees with previous reports that KIR44 is a KIR pseudogene (5).

The sequences and exon organizations of the new genes were compared with those of the known KIR (Figs. 1 and 2 and Table II). From this comparison, the four new KIR genes can be classified into three subgroups. The first one is constituted by KIR2DL5, which contains only two exons coding for Ig domains, and they are homologous to D0 and D2 of other KIR. Similarly to the situation in KIR2DL4 (16), these two exons of KIR2DL5 are separated by a 875-bp intron, in which no trace of an exon coding for the middle Ig domain was found after complete sequencing. Therefore, the protein encoded by KIR2DL5 would lack the D1 domain and is consequently predicted to be of the KIR2D type II, like KIR2DL4 (8). Despite the similarity in domain organization with KIR2DL4, the sequence of KIR2DL5 in exons 2–5 is marginally closer to type 1 KIR2D than to KIR2DL4 (Table II).

KIR15 has three exons for Ig-like domains and resembles type I KIR2D both in terms of the overall sequence similarity (Table II) and because of the presence of pseudogene 3. This nontranslated region, homologous to exon 3 of KIR3D (15), is shared by all type 1 KIR2D (28) and is characterized by the presence of a three-nucleotide deletion (Fig. 2). Expression of pseudogene 3 is prevented in some KIR2D, like KIR2DL1, by an in-frame stop codon, and this feature is shared by KIR15 (Fig. 2). In addition, KIR15 carries a one-nucleotide deletion in exon 4. The frame shift associated with this deletion creates a premature stop codon at exon 5, suggesting that KIR15 is probably a pseudogene.

Lastly, the organization of KIR44 and KIR48 resembles that of KIR3D because they contain three putative exons coding for Ig-like domains. The first of these has no premature stop codons, shows the canonical 285-bp length, and thus lacks the 3-bp deletion that is characteristic of pseudogene 3 (Fig. 2). However, the nucleotide sequence of KIR48 shows greater similarity to all KIR2D than to any KIR3D (Table II). This is not so evident for KIR44 because it shows 87.6 and 86.2% identity with KIR2DS2-NKAT5 and KIR3DL2-ε1.5, respectively. KIR44 is most closely related to KIR48, but they do not represent allelic variants of the same gene because two alleles of each were found in donor NV (Fig. 2). As already mentioned, no structural defects were found in exons 2–5 of KIR44 and KIR48.

Full-length cDNA from KIR2DL5.1 reveals an inhibitory KIR with D0-D2 configuration

Because KIR2DL5 is an expressed gene, we undertook experiments aimed at the complete characterization of its coding region. To do this, we used a two-step RACE strategy (19). First, we performed 3’-RACE using a forward primer (LFt196) that recognizes a specific change in exon 3 of KIR2DL5. From this PCR product, we obtained clones containing all the downstream exons of the gene, including the 3’-UTR and the poly(A) tail. These clones were sequenced and, using the information obtained, we designed a reverse primer (LRg1769) specific for a unique change in exon 3 of KIR2DL5. From this PCR product was obtained. The possibility that the KIR2DL5.2 sequence present in genomic DNA of donor NV was found to be expressed in cDNA. Twenty clones resulting from 5’-RACE, 3’-RACE, and standard RT-PCR cloning experiments contained the sequence labeled as KIR2DL5.1 in Fig. 2, whereas none corresponded to KIR2DL5.2. Furthermore, when cDNA of donor NV was tested with specific primers that should amplify KIR2DL5.2 but not KIR2DL5.1, no PCR product was obtained. The possibility that the KIR2DL5.2 sequence resulted from a PCR artifact was ruled out by PCR analysis of genomic DNA with different primer combinations. Specific PCR products derived from both KIR2DL5.1 and KIR2DL5.2 were obtained and identified by nucleotide sequencing. Therefore, donor NV seems to bear both an expressed and a nonexpressed donor NV was tested with specific

Table I. PCR amplification of exons 2-5 of KIR2DL5, KIR44, KIR48, and KIR15 from genomic DNA

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Forward</th>
<th>Reverse</th>
<th>Specificity</th>
</tr>
</thead>
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<tr>
<td>Fcon113 (intron 1-exon 2)</td>
<td>AGGGTTCTTCTTGCTGC</td>
<td>Rg410 (exon 3)</td>
<td>KIR2DL5, KIR15 (+ KIR3DL2 and several KIR2D)</td>
</tr>
<tr>
<td>Fcon113 (intron 1-exon 2)</td>
<td>AGGGTTCTTCTTGCTGC</td>
<td>Rg410 (exon 3)</td>
<td>KIR2DL5, KIR15 (+ KIR3DL2 and several KIR2D)</td>
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<tr>
<td>Fcon113 (intron 1-exon 2)</td>
<td>AGGGTTCTTCTTGCTGC</td>
<td>Rg410 (exon 3)</td>
<td>KIR2DL5, KIR15 (+ KIR3DL2 and several KIR2D)</td>
</tr>
</tbody>
</table>

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*The new genes were first identified in DNA segments spanning exons 2–3 (KIR2DL5 and KIR15), 3–4 (KIR15), and 4–5 (KIR44 and KIR48). In order to extend the sequence information on these new genes, an “exon walking” approach was used: PCR primers specific for unique sequences from each of these genes were combined with primers recognizing conserved or group-specific motifs of known KIR at adjacent exons. PCR products from primer combinations that gave a positive result were cloned and sequenced.

* Primer combinations that led to the discovery of the new genes.

* Sequence based on KIR2DL5.
FIGURE 2. Nucleotide sequences of the novel genes KIR44, KIR48, KIR2DL5, and KIR15. Putative exons 2–5 of KIR44a/b, KIR48a/b, KIR2DL5.1, KIR2DL5.2, and KIR15 were aligned with the corresponding regions of other KIR genes expressed by donor NV: KIR3DL1, KIR3DL2, KIR2DL4, KIR2DL1, and KIR2DS2 (KIR3DL1v, cl.5, KIR2DL4v1, KIR2DL1v, and NKAT5 variants, respectively (17)). The nontranslated “pseudoexon 3” regions of KIR2DL1v and KIR2DS2-NKAT5 (28) are shown in italics. Dashes (−) indicate identity with KIR3DL1, tildes (~) stand for unavailable sequences, and filled spaces (f) indicate nucleotide deletions. The area corresponding to exon 4 is blank in KIR2DL4 and KIR2DL5. Boundaries between exons are indicated by vertical bars.
nucleotide replacements in the third and fourth exons (Fig. 2): three are synonymous, the other two substitute Asp for Asn 152 and Ser for Gly 174 in the D2 domain (Fig. 3).

Full-length cDNA of KIR2DL5 comprises 1596 nucleotides and contains a 5'-UTR of 55 bp, an open reading frame of 1128 bp (375 aa), and a 3'-UTR of 413 bp. As was predicted from the partial sequence obtained from genomic DNA, the structural organization of KIR2DL5 is closest to that of KIR2DL4 (8) among human KIR (Figs. 1–3). In particular, the extracellular portions of both these KIR are made up of D0 and D2 domains, instead of the more common D1 and D2 of type I KIR2D. Also of note, KIR2DL4 and KIR2DL5 share a four-nucleotide deletion in exon 9 that distinguishes them from other KIR (not shown). The frame shift caused by this deletion gives KIR2DL4 and KIR2DL5 cytoplasmic tails that are longer (115 aa) than those of other inhibitory KIR (76–95 aa). Despite the structural similarities, it must be emphasized that KIR2DL5 cannot be an allelic form of KIR2DL4 because donor NV expresses two alleles of KIR2DL4 (17) as well as two forms of KIR2DL5.

The nucleotide and amino acid sequences of KIR2DL4 and KIR2DL5 are 89 and 79% identical, respectively. Although similar in sequence and domain organization, there are specific differences in the primary structures of KIR2DL4 and KIR2DL5 that could have functional importance. Whereas KIR2DL4 has a single immunoreceptor tyrosine-based inhibitory motif (ITIM) in its cytoplasmic region, KIR2DL5 has an additional ITIM separated by 24 residues, in which threonine is substituted for the first residue of the IV-X-Y-X2-L/V consensus motif (Fig. 3). Also, KIR2DL5 lacks the arginine residue present in the transmembrane region of KIR2DL4 (Fig. 3). This charged residue has been proposed (22) to mediate a possible interaction with the signal transducer protein DAP12 (23), which might render KIR2DL4 an activatory molecule in some circumstances. The presence of two ITIMs and the absence of charged residues in the transmembrane region point to KIR2DL5 being an inhibitory receptor.

KIR2DL5 is clonally expressed in NK cells and T cells

KIR2DL4 appears ubiquitously expressed by NK cells, whereas other KIR are differentially expressed in an apparently stochastic manner. To assess the expression of KIR2DL5, we examined a panel of NK cell clones from donor NV, all of which were known to express KIR2DL4 (17). Complementary DNAs from 96 clones were typed by PCR with KIR2DL5-specific primers. Twenty-two (23%) of the 96 clones expressed KIR2DL5, demonstrating that

Table II. Percentage of identity between the nucleotide sequences of the new KIR genes and those of other representative KIR genes expressed by donor NV*

<table>
<thead>
<tr>
<th>KIR3DL1</th>
<th>KIR3DL2</th>
<th>KIR2DL1</th>
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<th>KIR2DL4</th>
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</tr>
</tbody>
</table>

* Nucleotides 1–858 of the alignment shown in Fig. 2 were compared. Only one of the versions of each KIR2DL5, KIR44, and KIR48 are shown for simplification.
this KIR is not universally expressed like KIR2DL4. KIR phenotypes had been previously determined for 42 of the clones (17), and of these, we found four to be positive for KIR2DL5. As shown in Table III, KIR2DL5 expression does not seem to be linked to that of any other KIR. Although all four of the KIR2DL5-positive NK cell clones also expressed KIR2DL4 and KIR2DS2, the latter receptors were also seen in KIR2DL5-negative clones (not shown).

Analysis of 33 T cell clones selected for expression of known inhibitory receptors showed that seven expressed KIR2DL5 (not shown). These results show that KIR2DL5 is not ubiquitously expressed like KIR2DL4 but has a clonal distribution in NK cells and T cells that is analogous to that seen with other KIR.

**Alternative splicing of KIR2DL5 messenger RNA**

In addition to polynucleotide polymorphism, the KIR gene family produces additional variation through alternative splicing of mRNA (13, 15, 16). We found this mechanism to also operate on transcripts from the KIR2DL5 gene because analysis of eight cDNA clones obtained from RT-PCR showed only three of them contained all eight exons (Fig. 4). Interestingly, exon 2 was directly spliced to exon 6 (stem) in one of the clones; should this mRNA be translated, a protein containing a cytoplasmic tail and no extracellular domains would be generated. This structure resembles that of CD3-ζ or DAP12 (23), but unlike these molecules, any signal transmitted by this form of KIR2DL5 is predicted to be inhibitory rather than stimulatory. In addition to alternative splicing, variation was also observed in the length of the 3′-UTR of KIR2DL5. Whereas four of five clones obtained by 3′-RACE contained ~410-bp-long 3′-UTR similar to those reported for other KIR, the 3′-UTR extended in one clone for an additional 427 bp (not shown), indicating the potential use of a downstream polyadenylation signal.

**KIR2DL5 marks the B subset of KIR haplotypes**

Previously it was shown that individuals vary in the number of KIR genes they inherit, the differences being largely in the genes for noninhibitory KIR (14). Two types of KIR haplotype have been recognized, the A-haplotypes, which usually have KIR2DS4 as their only noninhibitory receptor, and the B-haplotypes, which have greater numbers and distinctive combinations of noninhibitory KIR. Individuals who are homozygous or heterozygous for B-haplotypes can be distinguished in Southern blot analysis by the presence of a 24-kb HindIII fragment, which is absent from individuals who are homozygous for A-haplotypes (14).

To assess the population distribution of KIR2DL5 and the other new genes, we studied a panel of genomic DNA samples isolated from unrelated individuals representing the three major ethnic groups. Eighteen individuals were typed by PCR with specific primers for KIR44, KIR48, and KIR15, and these three genes were found in all the samples. In contrast, KIR2DL5 was found in only fourteen of thirty-four donors who were typed for this gene. Comparison of the typing data with the previously performed Southern blot analysis (14) showed that the presence of KIR2DL5 perfectly correlated with the 24-kb HindIII fragment that is diagnostic of B-haplotypes (Table IV). Further typing of an additional 108 Caucassoid donors showed KIR2DL5 to be present in 56 (51.9%) of them. In these donors, 27 different KIR combinations were detected, which included ones having 2–6 noninhibitory KIR (not shown). KIR2DL5 was identified in individuals of Negroid, Caucasian, and Mongoloid origins, showing that it is not restricted to a particular ethnic group.

**Phylogenetic relationships of the new KIR genes**

To further assess the relationships between the new KIR and those previously described, a phylogenetic tree was built from a nucleotide sequence alignment using the neighbor-joining method (20). In the tree (Fig. 5), clear relationships with previously known KIR were demonstrated for KIR15 and KIR48. KIR15 is clearly grouped together with type I KIR2D, and KIR48 appears as an intermediate group between these and all other KIR. However, the phylogenetic relationship of KIR48 with type I KIR2D is less well-supported (bootstrap value, 68%). KIR44 is distinctive and does not group with any other known KIR family. Although both type II KIR2D, KIR2DL5 and KIR2DL4, were grouped together in the tree, the bootstrap value was relatively low (51%), a value consistent with the sequence similarities of KIR2DL5 with both KIR2DL4 and type I KIR2D (Table II). This suggests that, if KIR2DL4 and KIR2DL5 arose from the duplication of a common ancestral gene, they both have undergone considerable subsequent divergence with the possible involvement of recombination with other KIR genes. Orthologs of both KIR2DL4 and KIR2DL5 have
been found in the common chimpanzee (Pan troglodytes) (R.R., unpublished data) and show 98% and 97% nucleotide identity with their human counterparts, respectively (Figs. 3 and 5). This insertion indicates that divergence of KIR2DL4 and KIR2DL5 took place before the separation of these species some 5 million years ago (24).

**Discussion**

Previous studies have shown that KIR fall in three groups according to the number and organization of their extracellular Ig domains. One of these groups is defined by the D0-D2 configuration and, before this study, was only represented by KIR2DL4 (8). Here we have identified and characterized KIR2DL5, a second member of this family. The structural similarity between KIR2DL4 and KIR2DL5 is not limited to their extracellular domains but extends to the carboxy-terminal region of these molecules because both have distinctively longer cytoplasmic tails than other inhibitory KIR.

In addition to the expressed KIR2DL5.1 gene, we have characterized exons 2–5 of three other novel genes (KIR15, KIR44, and KIR48) and a variant of KIR2DL5 (KIR2DL5.2), none of which are transcribed in PBMC of the donor studied. Whereas KIR15 is likely a pseudogene of the type I KIR2D group, KIR44 and KIR48 show no structural anomalies in exons 2–5. Furthermore, the distinct nonexpressed KIRCI gene (18), of which KIR44a and KIR44b seem to be allelic variants, has also been characterized from genomic DNA and shown to contain a complete putative open reading frame with no premature stop codons. Therefore, it remains uncertain whether KIR genes like KIR44/KIRCI and KIR48 are pseudogenes or if they can be expressed in certain circumstances, e.g., particular individuals, tissues, stages of development, or physiopathological situations.

Despite the overall structural similarity of KIR2DL4 and KIR2DL5, differences in the primary structures of these receptors suggest that they could be functionally distinct. Whereas KIR2DL4 has been reported to recognize HLA-G (25), the 35 aa differences between the extracellular domains of KIR2DL4 and KIR2DL5 (20 differences in D0 and 15 in D2; Fig. 3) raise the possibility that the latter recognizes a different ligand, which has yet to be determined. Also, although KIR2DL4 shows some unusual characteristics among inhibitory KIR, such as a single intracellular ITIM and a charged residue in the transmembrane region, KIR2DL5 shows all the features of a typical inhibitory KIR, including two ITIMs and no charged residue in the transmembrane region.

Additional differences between KIR2DL4 and KIR2DL5 become apparent when their population distribution and cell expression are compared. KIR2DL4 is a ubiquitous receptor both in the sense that every individual possesses the gene (14) and that all NK cells seem to express it (17, 25). On the contrary, KIR2DL5 contributes to KIR diversity within the population because it is present only in some individuals, notably those having B-haplotypes that bear noninhibitory KIR other than KIR2DS4. In addition, the complexity of the KIR repertoire of NK cells is even greater than...
previously considered (17) because KIR2DL5 is expressed in only a fraction of NK and T cells with no apparent combination with any other KIR. Thus, the number of possible random combinations of KIR expressed by NK and T cells is further increased in individuals expressing KIR2DL5. The different patterns of KIR2DL4 and KIR2DL5 expression suggest that although the former probably has an essential role in NK cell physiology, KIR2DL5 may have a more subtle, complementary or even redundant function.

Previously, donor NV has been shown to be heterozygous for KIR2DL4 (17). In this study, we find him to bear two variants of three additional KIR genes (KIR2DL5, KIR44, and KIR48), further illustrating the polymorphism of the KIR system. The two variants of KIR2DL5 differ by only five nucleotide substitutions in exons 2–5 and, although one of the versions (KIR2DL5.1) is transcribed, the other (KIR2DL5.2) remarkably is not. Because the observed sequence differences between KIR2DL5.1 and KIR2DL5.2 are minor in nature (Figs. 2 and 3), they cannot explain the lack of expression of the latter, which may be due to structural defects in uncharacterized regions of KIR2DL5.2. However, the possibility that the lack of KIR2DL5.2 expression is due to some sort of specific down-regulation is also worth consideration. Of potential relevance in this regard is that paternally imprinted genes.


