Cutting Edge: KIR3DS1, a Gene Implicated in Resistance to Progression to AIDS, Encodes a DAP12-Associated Receptor Expressed on NK Cells That Triggers NK Cell Activation


*J Immunol* 2007; 178:647-651; doi: 10.4049/jimmunol.178.2.647
http://www.jimmunol.org/content/178/2/647

---

**References**

This article cites 24 articles, 10 of which you can access for free at:
http://www.jimmunol.org/content/178/2/647.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Cutting Edge: KIR3DS1, a Gene Implicated in Resistance to Progression to AIDS, Encodes a DAP12-Associated Receptor Expressed on NK Cells That Triggers NK Cell Activation


The killer cell Ig-like receptor (KIR) gene, KIR3DS1, has been implicated in slowing disease progression in HIV infection; however, little is known about its expression, function, or ligand specificity. Using retrovirally transduced NK cells and peripheral blood NK cells from KIR3DS1-positive donors we assessed expression of this gene by flow cytometry and its function by in vitro assays measuring KIR3DS1-induced cell-mediated cytotoxicity and cytokine production. In the present study, we demonstrate that KIR3DS1 is expressed on peripheral blood NK cells and triggers both cytotoxicity and IFN-γ production. Using cotransfection and coimmunoprecipitation, we found that KIR3DS1 associates with the ITAM-bearing adapter, DAP12. Soluble KIR3DS1-Ig fusion proteins did not bind to EBV-transformed B lymphoid cell lines transfected with HLA-Bw4 80I or 80T allotypes, suggesting that if KIR3DS1 does recognize HLA-Bw4 ligands, this may be peptide dependent. The Journal of Immunology, 2007, 178: 647–651.

Naturally occurring genetic differences between individuals influence the clinical outcome of HIV-1 infection. The impact of these differences ranges from slower disease progression to AIDS to resistance to infection. Recently, Martin and colleagues found that a killer cell Ig-like receptor (KIR) gene, KIR3DS1, confers protection from disease progression to AIDS in HIV-1-infected adults (1). Independently, similar findings were made by other researchers who found slower disease progression in HIV-1-infected patients with both KIR3DS1 and HLA-B*57 supertype alleles (2).

Despite accumulating evidence that KIR3DS1 plays a role in the clinical outcome of HIV-1 infection, little is known about its expression, function, or ligand specificity. This gene was first identified from sequence analysis of KIR gene cDNA transcripts isolated from human NK cells (3). KIR3DS1 encodes a predicted type 1 transmembrane protein of ~50 kDa with three extracellular domains and secures as an allele of the KIR gene, KIR3DL1 (4–6). Although KIR3DL1 and KIR3DS1 share 97% amino acid homology, they differ markedly in their cytoplasmic domains. KIR3DL1 has a longer cytoplasmic tail that contains ITIM, which permits signaling to inhibit NK cell responses. In contrast, KIR3DS1 lacks ITIM in its cytoplasmic tail and has a positively charged residue in its transmembrane domain. Other KIR with a short cytoplasmic tail and a positively charged transmembrane domain residue, such as KIR2DS1 and KIR2DS2, recruit an ITAM-bearing adaptor molecule, DAP12, for stimulatory signaling (7–9); however, the function and signaling requirements for KIR3DS1 remain undetermined.

The ligand for KIR3DS1 has not been investigated previously. KIR3DL1, which has specificity for HLA-B allotypes with a Bw4 public epitope (10), generates the strongest inhibitory response to allotypes with an isoleucine at position 80 (80I), such as members of the B*57 superfamily (11, 12). Based on these findings, our objective was to determine the expression, function, and ligand specificity of KIR3DS1 as a foundation for understanding its role in delaying HIV-1 disease. In the present study, we use a previously described Ab against KIR3DL1 that cross-reacts with KIR3DS1 to investigate the expression, function, and ligand specificity of KIR3DS1.
Materials and Methods

Cell lines, human donors, and mAbs

Cell lines used were: NKL cells (a gift from M. Robertson, Indiana University, Indianapolis, IN), IL-3-transduced Ba/F3 (a gift from S. Tangye, Centenary Institute, Sydney, Australia), 721.221 HLA-transfectants expressing −B*5701 (a gift from C. Lopez-Larrea, Hospital Central de Asturias, Oviedo, Spain), or other HLA transfectants (a gift from P. Parham, Stanford University, Palo Alto, CA), and KIR3DL1*002-transfected Jurkat T cells (a gift from P. Parham and M. Pandol). KIR3DS1+* cell lines were generated by cloning KIR3DS1+*1 (GenBank accession no. AF020244) cDNA (a gift from P. Parham) into retroviral vectors (13), containing the human CD8 leader segment, followed by either a Flag or V5 epitope at the N terminus. PBMC were obtained from healthy human donors by informed consent under an Institutional Review Board approved protocol and were characterized for KIR genotype by PCR-sequence specific primer (SSP) genotyping as described previously (14). Polyclonal NK cells were generated in vitro in the presence of IL-2 as described previously (15). mAbs were obtained from BD Biosciences with the following exceptions: anti-KIR3DS1/L1 (clone Z27) (Beckman Coulter), anti-Flag (clone M2; Sigma-Aldrich), anti-myc (clone 9E11; Sigma-Aldrich), anti-V5 (Serotec), anti-NKG2D (clone 149810; R&D Systems), and PE-conjugated goat anti-mouse IgG (Jackson Immunoresearch Laboratories). Fluorescent staining was analyzed by using a FACSCalibur or a FACScan (BD Biosciences) and FlowJo analysis software, version 6.3 (Tree Star).

Adaptor molecule association studies and functional assays

Adaptor molecule associations, immunoprecipitations, Western blotting, and Ab-induced redirected cytotoxicity assays were performed as described previously (16). For plate-bound mAb stimulation 2.5 × 10⁴ effector cells per well were cultured for 24 h in 96-well Nunc Maxisorp plates (eBioscience) coated with 10 μg/ml of the appropriate mAb and assessed by a human IFN-γ ELISA kit (eBioscience).

Ligand recognition assays

Soluble KIR-IgG/A-Fc fusion proteins were generated from a chimera of the extracellular domains of KIR3DS1 or KIR2DS1 with the SLAM leader sequence, a mutated IgG to eliminate FeR binding, and an IgA tail piece in the pMCI1s vector as described previously (17). Simply Cellular anti-human IgG beads (Bangs Laboratories) were used in the determination of Ig Fc fusion protein recognition by anti-KIR mAbs. We generated KIR3DS1 reporter cells by transduction of NFAT-LacZ 2B4 T cell hybridoma cells (a gift from N. Shastri, University of California, Berkeley, CA) with retroviral plasmids containing DAP12 and the extracellular and transmembrane domains of KIR3DS1 with the cytoplasmic tail of CD3ζ. To determine β-galactosidase activity we measured light absorption at OD₅₉₅ using the colorimetric substrate, chlorophenol red galactoside on a Versamax ELISA plate reader (Molecular Devices).

Results and Discussion

KIR3DS1 is expressed on transduced NKL cells and NK cells in peripheral blood

To assess the function and expression of KIR3DS1, we stably transduced a transformed NK cell line, NKL, with an N-terminal Flag epitope-tagged KIR3DS1 cDNA. NKL cells lack KIR expression (with the exception of KIR2DL4) (18), but do express DAP12. We found that KIR3DS1 was expressed on the cell surface of the transduced cells, but not on untransduced cells as assessed by anti-Flag mAb staining (Fig. 1A). Its expression was also detectable by the clone Z27 mAb, an Ab that has been described previously to recognize KIR3DL1 (19). We also confirmed that this mAb recognized KIR3DL1 expressed on stable transfectants of Jurkat T cells (Fig. 1B). Unexpectedly, we found that recognition of KIR3DS1 was unique to this mAb because another mAb with anti-KIR3DL1 specificity, clone DX9 mAb, did not recognize KIR3DS1. We tested Z27 mAb recognition of KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DS2, KIR2DS4, and KIR2DL5 using transfectants expressing these other KIR, respectively, and found no recognition of these KIR (data not shown). To further assess expression of KIR3DS1 we subsequently evaluated freshly isolated NK cells from healthy human donors. Using donors, whom were genotyped for the presence of KIR3DS1 and KIR3DL1 by PCR-SSP typing, we discovered that the frequency of KIR3DS1+/− NK cells in peripheral blood could be determined by the combination of Z27 and DX9 mAb staining (Fig. 1C and D). In one donor, who was KIR3DS1 homozygous, we found that ~30% of the CD3+CD56+ NK cells expressed KIR3DS1. Also in this donor ~0.6% of the CD3+CD56+ T cells expressed KIR3DS1 (data not shown). These findings establish that KIR3DS1 is expressed on freshly isolated NK cells and a subset of T cells in peripheral blood.

KIR3DS1 associates with DAP12

Based on our discovery that KIR3DS1 was expressed at the cell surface in NK cells, we addressed the question of whether it also associated with an adaptor molecule. Using a mouse B cell line, Ba/F3, we cotransfected a plasmid containing Flag epitope-tagged KIR3DS1 with and without plasmids containing the adaptor molecules DAP10 or DAP12 (Fig. 2A). As a control, we assessed transfection of a plasmid containing KIR2DS1, an activating KIR that associates with DAP12 but does not require this adaptor molecule for its cell surface expression. Whereas there was minimal expression of KIR3DS1 in the absence of DAP12, significant up-regulation of surface expression occurred in the presence of DAP12 for both KIR2DS1 and KIR3DS1 (Fig. 2A). In a similar manner, we also evaluated coexpression with DAP10, which associates with the activating receptor NKG2D. Unlike NKG2D, expression of KIR3DS1 was not increased significantly on the cell surface in the presence of DAP10. Furthermore, to independently confirm the association of KIR3DS1 with DAP12 we performed Western blot analysis of immunoprecipitated proteins (Fig. 2B).
this analysis, we generated an N-terminal V5-epitope tagged KIR3DS1 construct and expressed it in BaF/3 cells that have been transduced to stably express DAP12. Immunoprecipitation of V5-KIR3DS1 transfected cells with an anti-DAP12 mAb coimmunoprecipitated an anti-V5 mAb-reactive 50-kDa protein, which was consistent with V5-epitope tagged KIR3DS1 (Fig. 2B). Immunoprecipitation of this protein did not occur with a control mAb; thus, this association was specific. Altogether, from the cotransfection and coimmunoprecipitation experiments we conclude that KIR3DS1 associates with DAP12 and that this association enhances its cell surface expression.

**KIR3DS1 triggers cytolysis and IFN-γ production**

Based on previous studies implicating a stimulatory role for KIR that associate with DAP12 (e.g., KIR2DS1 and KIR2DS2) (7–9), we tested the hypothesis that KIR3DS1 functions as an activating receptor. Using both Flag-KIR3DS1-transduced NKL cells and cultured KIR3DS1null NK cells derived from peripheral blood, we determined the function of KIR3DS1 by two independent methods: 1) mAb-redirected lysis and 2) plate-bound mAb stimulation of cytokine secretion (Fig. 3). In the redirected lysis assays, only anti-Flag (clone M2)- or anti-KIR3DS1/L1 (clone Z27)-specific mAbs, but not isotype-matched control mAbs, triggered lysis by NK cells that expressed Flag-KIR3DS1 (Fig. 3A). In contrast, these mAbs did not trigger lysis by wild-type NK cells. These results were not due to intrinsic differences in lytic capacity since we did not observe differences in their response to stimulation through another activating receptor, 2B4 (Fig. 3A).

To confirm that these responses were not limited to transduced cells, we conducted similar assays with NK cells derived from peripheral blood. In these assays, we used cultured, polyclonal NK cells derived from KIR3DS1-genotyped donors whom we had characterized previously (Fig. 1C). Compared with an isotype-matched control mAb, the anti-KIR3DS1/L1 mAb (clone Z27) triggered greater lysis only with NK cells from the KIR3DS1 homozygous donor (Fig. 3B), which is consistent with our previous findings using KIR3DS1-transduced NKL cells. In contrast, the addition of the mAb Z27 reduced lysis by NK cells from donors that possessed KIR3DL1. The difference between these two donors in the lysis with the isotype-matched control Ig treatment suggests a higher level of background lysis among the polyclonal population of donor C cells. In summary, we conclude that KIR3DS1 activates cytolysis in KIR3DS1-transduced NKL cells, as well as NK cells derived from peripheral blood that express endogenous KIR3DS1.

In addition to cytolysis, cytokine production is another critical effector function of NK cells in response to virally infected cells. We assessed the ability of the Z27 mAb to elicit IFN-γ production. Using plate-bound mAbs, KIR3DS1 was as effective as another activating receptor, 2B4, in stimulating IFN-γ production by KIR3DS1-transduced NKL cells (Fig. 3C). Flag-KIR3DS1-expressing NKL cells produced significantly more IFN-γ than wild-type NK cells in response to receptor cross-linking by anti-Flag mAb (p < 0.01, Student’s t test). The amount of IFN-γ production was comparable to that induced by anti-2B4 mAb. We observed similar results using the anti-KIR3DS1/L1 mAb Z27 (data not shown). Notably, we did not observe any effect on wild-type NK cells with the addition of anti-Flag mAb compared with unstimulated cells.
Thus, we conclude that KIR3DS1 is effective in eliciting both IFN-γ production and cytolytic activity.

To independently validate these findings, we assessed the responses of KIR3DS1^pos^ NK cells derived from peripheral blood. Using polyclonal, cultured NK cells derived from KIR3DS1-*genotyped individuals described above (Figs. 1C and 3B), we performed similar plate-bound mAb stimulation assays. In these assays, anti-KIR3DS1/L1 mAb-stimulated NK cells from the KIR3DS1 homozgyous donor produced significantly more IFN-γ than unstimulated NK cells (p < 0.01, Student’s t test) (Fig. 3D). In comparison, IFN-γ production by mAb-stimulated NK cells from the KIR3DS1null donor did not differ from unstimulated NK cells (p > 0.05). This lack of response was not due to a general inability to respond to stimulation. Stimulation through CD16, another activating NK cell receptor, by anti-CD16 mAb yielded equivalent IFN-γ production by NK cells from the KIR3DS1null donor compared with the KIR3DS1 homozygous donor (data not shown). Thus, our data are consistent with KIR3DS1 functioning as a stimulatory receptor in triggering both IFN-γ release as well as cytotoxicity.

KIR3DS1 does not recognize HLA-Bw4 on 721.221 transfectants

With such a high homology (97%) to the inhibitory KIR3DL1 receptor (3), we hypothesized that KIR3DS1 would share the same ligand specificity. The previously described association between KIR3DS1 and HLA-Bw4 80I with protection from AIDS progression (1) suggested that KIR3DS1 may interact directly with HLA-Bw4 80I allotypes, such as HLA-B*5701. To test this hypothesis, we generated soluble KIR-Ig-Fc fusion proteins of KIR3DS1 and assessed HLA specificity by staining HLA-transfectants of 721.221 cells, a HLA class I-deficient B-lymphoblastoid cell line. As a positive control, we also measured binding using KIR2DS1-Ig Fc fusion proteins. Both the KIR3DS1- and KIR3DS1-Ig Fc fusion proteins were recognized by their respective anti-KIR specific mAb (Z27 and HP3E4, respectively) in vitro (Fig. 4A), suggesting that these proteins were folded properly, and were assessed at equivalent concentrations (Fig. 4B). As expected, the KIR2DS1-Ig Fc proteins recapitulated previously established KIR2DS1/L1-specificity for HLA-Cw*0401 (Fig. 4C) (20). This staining of the HLA-Cw*0401pos cells was not due to higher class I expression, as the other 721.221-transfectants did not differ in HLA expression (p > 0.01, ANOVA). Surprisingly, the soluble KIR3DS1-Ig Fc protein did not bind either to 721.221 cells expressing HLA-B*5701 (Fig. 4C) or other Bw4 (B*5801 (80I), B*2705 (80T)) or Bw6 (B*1502) allotypes evaluated (data not shown). Previously, a mouse T cell line (BWZ), which contained an NFAT reporter construct and had been transfected with DAPI2, was used to identify the ligand for Ly49H, a mouse NK receptor analogous to stimulatory KIR (196; Ref. 17). Here we used a similar approach to assess recognition of B*5701 by KIR3DS1. Using BWZ reporter cells expressing a chimeric KIR3DS1 receptor with a CD3ζ cytoplasmic tail we found that plate bound anti-KIR3DS1 mAb stimulation triggered strong responses not observed with reporter cells lacking KIR3DS1 (Fig. 4D). However, coculture with B*5701-721 cells elicited responses only marginally greater than those to HLA-null-721 cells (Fig. 4E). These responses were not inhibited by an anti-MHC class I mAb that abrogates inhibitory KIR3DL1 and HLA-Bw4 interactions (10). From these results, we conclude that KIR3DS1 does not recognize HLA-Bw4 under the conditions evaluated.

Although we did not observe convincing HLA binding by KIR3DS1, we cannot exclude the possibility that binding occurred below our detection limits. Since we did observe significant binding by soluble KIR2DS1 proteins, if KIR3DS1 does interact with HLA-Bw4 the binding might be of lower affinity than KIR2DS1 with HLA-C. Alternatively, HLA recognition by KIR3DS1 may require additional factors, such as the presence of specific peptides, which are presented in the HLA-B peptide-binding groove. This explanation is feasible based on previous studies that have shown a considerable influence of the HLA-bound peptide on KIR recognition (21, 22). We speculate that detectable KIR3DS1 binding of HLA-B-peptide complexes may only occur under special circumstances, such as during HIV infection. KIR recognition of HLA-associated virally encoded peptide complexes has precedence in the recognition of HLA-associated EBV-encoded peptide complexes (23). Physiologically, this mechanism of recognition would be ideal.
for the detection of pathogens, without the risk of autoimmunity. Our data do not exclude the possibility that KIR3DS1 directly recognizes a virally encoded protein as has been previously described for Ly49H (17) or indirectly provides protection from HIV disease progression. Recently, Qi et al. (24) found that the KIR3DS1 gene was associated with protection from opportunistic infections. Furthermore, as an allele of KIR3DL1, KIR3DS1 may alternatively effect slower disease progression through reduced KIR3DL1 expression, a gene dosage effect. Additional studies using HIV-infected cells and/or HIV-encoded peptides are required to further investigate the mechanisms that determine KIR3DS1 ligand specificity and reveal its role in slower HIV disease progression.

Acknowledgments

We thank Susan Watson for assistance and Warner Greene and Marcelo Pando-Rigal for valuable discussions.

Disclosures

The authors have no financial conflict of interest.

References


