Inhibition of natural killer cell activation signals by killer cell immunoglobulin-like receptors (CD158)

Summary: The killer cell immunoglobulin-like receptor (KIR) family includes receptors that bind to HLA class I molecules on target cells and inhibit natural killer (NK)-cell cytotoxicity, and receptors such as KIR3DL7 with no known ligand and function. Inhibitory KIR recruit the tyrosine phosphatase SHP-1 to block signals transduced by any one of a number of activation receptors. Inhibition of overall protein tyrosine phosphorylation by SHP-1 during binding of KIR to MHC class I on target cells is selective, suggesting that a limited number of substrates are dephosphorylated by SHP-1. We have chosen to study KIR inhibition as it occurs during binding of KIR to MHC class I on target cells, despite the technical limitations inherent to studies of processes regulated by cell contact. KIR binding to MHC class I on target cells inhibits tyrosine phosphorylation of the activation receptor 2B4 (CD244) and disrupts adhesion of NK cells to target cells. Inhibition of proximal events in NK activation may increase the availability of NK cells by liberating them from non-productive interactions with resistant target cells. As the receptors and the signaling pathways that induce NK cytotoxicity are not fully characterized, elucidation of the inhibitory mechanism employed by KIR may provide insight into NK activation.

Introduction

An important function of inhibitory killer cell immunoglobulin-like receptors (KIR) is to provide tolerance to self (1–3). Hematopoietic cells are particularly sensitive to lysis by natural killer (NK) cells and require recognition of their own MHC class I by NK inhibitory receptors in order to be spared. Target cells that are eliminated by NK cells in vivo may include cells infected by viruses that interfere with MHC class I expression, and tumor cells that have lost expression of MHC class I. However, the role of inhibitory KIR has yet to be fully evaluated in vivo. The presence of immunoreceptor tyrosine-based inhibition motifs (ITIM) in the cytoplasmic tail of inhibitory KIR and the recruitment of tyrosine phosphatases by phosphorylated ITIM, leading to inhibition of NK-cell activation, has become a paradigm for the negative control of cellular responses by an increasingly large number of receptors (4–6). Therefore, the mechanism of NK inhibition by
Table 1. KIR nomenclature

| KIR  | CD  | Signal  | HLA Igand |
|------|-----|---------|-----------|
| 2DL1 | 158a| Inhibitory | C group 2 |
| 2DS1 |     | Activating | C?        |
| 2DL2 | 158b| Inhibitory | C group 1 |
| 2DL3 | 158b| Inhibitory | C group 1 |
| 2DS2 |     | Activating | C?        |
| 2DL4 |     | Potential for both | G         |
| 2DL5 |     | Potential for inhibition | ?        |
| 2DS4 |     | Activating | ?         |
| 2DS5 |     | Potential for activation | ?       |
| 3DL1 |     | Inhibitory | Bw4       |
| 3DS1 |     | Potential for activation | ?       |
| 3DL2 |     | Inhibitory | A3, A11   |
| 3DL7 |     | Potential for inhibition | ?       |

* 2D and 2D designate KIR with two or three Ig domains, respectively. L and S refer to long and short cytoplasmic tails, respectively. The centromeric to telomeric arrangement of genes is KIRC1 (3DL7) – 2DL2 or 2DL3 – 2DL1 – 2DL4 – 3DL1 – [2DL5 – 2DS5 – 2DS1] – 2DS4 – [2DS2] – 3DL2. Genes in square brackets are absent in some KIR haplotypes (8).

* A complete CD158 nomenclature is under development.

KIR is likely to provide insights into negative regulatory controls in diverse biological systems. This review attempts to summarize the current state of knowledge on the mechanism by which KIR inhibits NK cytotoxicity.

The group of receptors encoded in a gene cluster at the human chromosomal location 19q13.4 (7, 8) was originally called killer cell inhibitory receptors, since it included receptors such as p58 (9) and NKB1 (10), which block activation upon recognition of HLA-C and HLA-B molecules on target cells, respectively. The acronym KIR was proposed to identify this receptor family (11). Killer cell Ig-like receptors has become a more suitable term for the family (12) and distinguishes KIR from lectin-like receptors with similar inhibitory function, such as CD94/NKG2 and mouse Ly49. CD158 was proposed at the last Human Leukocyte Differentiation Antigen Meeting as a designation for the entire KIR family. Table 1 lists the KIR family members and the corresponding CD158 subsets.

The KIR family

Inhibitory members of the KIR family include receptors with two Ig domains (KIR2D, domains D1 and D2) and a long (L) cytoplasmic tail. KIR2DL1 (CD158a) is specific for group 2 HLA-C allotypes defined by asparagine at position 77 and lysine at position 80. KIR2DL2 and 2DL3 (grouped together as CD158b) are specific for group 1 HLA-C allotypes with a serine at 77 and asparagine at 80. The specificity of 2DL2 and 2DL3 for HLA-C group 1 and of 2DL1 for HLA-C group 2 is not completely tight, as recognition of the opposite HLA-C group has also been observed in functional assays and by direct binding (13, 14). HLA-C has an obvious important role in providing protection from NK cells because every HLA-C allotype is recognized by an inhibitory KIR. KIR3DL1 has three Ig domains (KIR3D, domains D0, D1, and D2) and a long cytoplasmic tail. 3DL1 is specific for B allotypes that carry the Bw4 serological determinant. All of these inhibitory receptors contribute to NK tolerance to self through the recognition of their specific MHC class I ligands on target cells (3). The receptor KIR3DL2 has binding affinity for HLA-A3 (15). Inhibition of 3DL2+ NK clones by HLA-A3 and HLA-A11 on target cells has been observed (16) but a contribution of 3DL2 in tolerance to self has not been readily apparent (3). Expression of inhibitory KIR on NK cells is not dictated by the HLA haplotype of the individual. All KIR genes are expressed in NK cells regardless of whether the individual has corresponding MHC class I ligands or not. Each NK cell expresses a small repertoire of about two to six KIR (3).

Several KIR with two Ig domains and short (S) cytoplasmic tails, such as 2DS1 and 2DS2, have activating function (12). KIR2DS associate with DAP12 subunits via a charge interaction in the transmembrane region (17). DAP12, containing an immunoreceptor tyrosine-based activation motif (ITAM), initiates a src-kinase and syk/ZAP70 kinase-dependent activation cascade. Although direct binding of 2DS1, 2DS2, and 2DS4 to HLA-C is almost undetectable (13, 14, 18–20), functional experiments with NK clones and T-cell clones suggest that an activation signal can be delivered through recognition of HLA-C on target cells (21, 22). A proposed function for such receptors is to promote killing of tumor cells or virus-infected cells that have selectively lost expression of a protective MHC class I. Sensitivity to lysis by NK cells may actually be increased if the remaining class I allotypes are recognized by an activating KIR2DS (6, 23).

KIR2DL4 has the potential for both inhibitory and activating function, as it contains an ITIM in the cytoplasmic tail and a charged amino acid residue (arginine) in the transmembrane region, indicative of association with another molecule. Soluble recombinant 2DL4 binds to cells expressing HLA-G, suggesting a role of 2DL4 in pregnancy (24, 25). HLA-G is expressed primarily on trophoblast cells that invade the decidua at the maternal–fetal interface during early pregnancy. Abundant maternal NK cells in the decidua encounter invading trophoblast cells (26). The unique D0–D2 domain
configuration of 2DL4 is also found in 2DL5, a KIR whose ligand and function are unknown (27).

KIR3DL7
KIRCl, the first gene at the centromeric end of the KIR complex, encodes a putative inhibitory receptor with three Ig domains (8, 28). Transcription of KIRCl has not been detected at the RNA level. Using nested PCR with RNA from peripheral blood, we have isolated full-length cDNA clones corresponding to the deduced mRNA of KIRCl (N. Wagtmann, V. Renard, M. Sandusky, M. Peterson, E. O. Long, unpublished; GenBank accession AF352324). Expression of KIRCl may be very low because amplified cDNA was not easily detected except after using internal primers in a second round of PCR. As predicted from the gene sequence (28), the cDNA encodes a KIR with three Ig domains but without the stem that connects the D2 domain with the transmembrane domain of all other KIRs. The cytoplasmic tail carries the first ITIM and stops at the level of the second ITIM. In accordance with a unified human and chimpanzee KIR nomenclature proposed by Parham (29), the KIRCl product should be called 3DL7. (The names 3DL to 3DL6 have been assigned to chimpanzee KIR that have no human orthologs.) The 3DL7 sequences of three individuals, deduced from the KIRCl gene sequenced by Torkar et al. (28) (accession AF072407 and AF072410) and by the Lawrence Livermore National Laboratories (http://www-bio.llnl.gov/bbrp_genome_genome.html) (accession AC006293), and from the cDNA described here, differ from each other by two to five amino acids, indicating some polymorphism in this gene.

3DL7 contains some unique amino acids not found in any other KIR of humans and chimpanzees (Fig. 1). Otherwise, 3DL7 resembles a patchwork of sequences found in other KIR. The D0 domain is very similar to that of chimpanzee (Pt) KIR3D receptors, in particular Pt-3DL4, which shares specificity for group 2 HLA-C with KIR2DL1 (29). The D0 domain shares sequences with 2DL4, Pt-3DL4, and 3DL1, and the D1 domain has sequences found predominantly in 3DL2 and 3DL1. The D2 domain is more mixed and includes stretches of sequences identical to 2DS4 and 2DL4. Rajalingam et al. (30) have proposed that KIRCl may correspond to an ancestral gene from which several other KIR genes were derived during evolution. Despite the presence of a conserved
Inhibition of NK-cell activation signals by KIR

A fundamental question on the mode of inhibition by KIR is whether a central step in the NK activation pathway is specifically blocked or whether KIR can target multiple signals for inhibition. The complete block of cytotoxicity, lymphokine production, and of Ca\(^{2+}\) flux exerted by KIR is compatible with both hypotheses. At the cellular interface of the NK–target cell conjugate, the clustering of KIR may lead to its tyrosine phosphorylation by an src kinase within the activation pathway. However, the specific requirements for KIR clustering upon binding to MHC class I and the resulting KIR phosphorylation are not known. Furthermore, phosphorylation of KIR by a kinase that is involved in the activation pathway seems to put KIR at a disadvantage. How can KIR inhibit a tyrosine kinase-dependent signal when its own tyrosine phosphorylation is a prerequisite for inhibition? Placing KIR downstream of the initial signaling event in NK activation inevitably delays its ability to turn off the signal. Such a delay could be overcome if the critical site of KIR inhibition is further downstream in the signaling pathway for activation. However, existing evidence maps KIR function at a fairly proximal step of the activation signal (31–34).

Experimental approaches to study inhibition by KIR

The ideal setting to study how KIR exerts its inhibitory effect would be with normal NK cells receiving physiological signals from soluble mediators (e.g. cytokines, chemokines) while they contact other cells that express a ligand for KIR. Relevant target cells would be those that are sensitive to lysis by NK cells unless protection by MHC class I is provided. Such a setting is difficult to reproduce and would reduce the range of feasible experiments. Nevertheless, normal NK cells are easy to obtain, and they can be expanded and cloned in vitro. Biochemical analysis of the negative signal delivered by KIR during NK–target cell contacts is complicated by the signals and proteins contributed by target cells and by the lack of synchrony in the interaction between cells. To reconstitute inhibition by MHC class I on target cells, the class I-negative mutant cell line 721.221 transfected with specific class I genes is often used. It is therefore possible to observe under controlled conditions the outcome of KIR engagement by MHC class I on target cells (Fig. 2A). Early experiments using such a system established that the block exerted by KIR lies upstream of Ca\(^{2+}\) flux (31, 32).

Much progress in the characterization of NK receptors was achieved by use of a redirected lysis assay, also referred to as reverse antibody-dependent cellular cytotoxicity (Fig. 2B). FcR\(^{+}\) target cells (e.g. mouse P815 cells) are used to cross-link receptors on NK cells with mAbs that bind NK receptors. mAbs specific for activation receptors induce lysis of P815 by NK cells, whereas mAbs specific for inhibitory receptors can inhibit the lysis triggered by mAbs specific for activation receptors. However, some limitations also apply to this system. The presence of mouse target cells complicates biochemical analysis of NK signaling components, which are often conserved among distinct species. Furthermore, the use of Abs to cross-link receptors may not reproduce important parameters of receptor–ligand interactions during cell contact, such as density, topology, and specific localization in the membrane.

Antibodies can be used to bypass the complications inherent to cell mixing experiments. A strong activation signal is delivered to NK cells simply by cross-linking CD16. This
signal is inhibited by co-cross-linking an inhibitory receptor (Fig. 2C). This approach has been applied to study the effect of inhibitory KIR on CD16 signaling. However, results obtained by this approach can differ from those of NK–target cell mixing experiments.

Phosphorylation and clustering of KIR

The inhibition by KIR acts only when the receptor is engaged in close proximity to the activation signal. Independent cross-linking of KIR and of the FcεRI receptor on a transfected rat basophil cell line did not impair the activation induced by IgE, whereas co-cross-linking the two receptors did (35). Free SH2 homology 2 (SH2)-containing tyrosine phosphatase (SHP)-1 has a low catalytic activity because its own SH2 domains blocks the catalytic site (36). Therefore, binding of the SH2 domains of SHP-1 to phosphorylated KIR releases the inhibition and induces dephosphorylation of substrates that are in close proximity to KIR. The need to co-ligate KIR with an activation receptor in order to achieve inhibition may serve two purposes. One, as mentioned, is to bring active SHP-1 to the site where activation signals are transmitted. The second is to move KIR to the site of activation where active src kinases will phosphorylate the ITIM.

Several models can account for the ability of KIR to inhibit an src kinase-dependent signaling cascade even though it requires an src kinase to phosphorylate its own cytoplasmic tail. First, inhibitory KIR may be constitutively phosphorylated. In that case, inhibition would still depend on KIR clustering at the site of activation upon binding to MHC class I on the target cell. Second, KIR may be associated with a tyrosine kinase. KIR phosphorylation would be induced after activation of the kinase upon KIR clustering, much like ITAM-dependent activation receptors. The third model is that KIR phosphorylation is executed by those kinases that are initiating the NK activation signal. In that case, inhibition by KIR would have to either be extremely fast in order to stop a signaling cascade that has already started, or target a downstream effector of the activation pathway. None of these models has been excluded so far, although the lack of detectable KIR phosphorylation in the absence of co-igation with activation receptors supports the third model. Furthermore, KIR binding to HLA-C on target cells results in extensive clustering at the cell interface, a property that may accelerate KIR’s ability to stop the activation signals (37). Unexpectedly, out of several inhibitors tested, only Zn2⁺ chelators, such as 1,10-phenanthroline, reduced the extent of KIR clustering. The propensity of KIR to cluster may contribute to its inhibitory function by concentrating KIR near activating receptors before full induction of triggering signals.

Earlier work had established that Zn2⁺ binding to KIR through histidine residues plays a role in the inhibitory function of KIR (38, 39). More recently, biochemical studies with soluble 2DL1, purified after expression in bacteria, revealed a dimerization of 2DL1 induced by Zn2⁺ and by the related metals Cu2⁺ and Co2⁺ (14, 40). Metal-induced dimers of KIR bound to HLA-C with greater affinity or avidity than did KIR monomers. Surface plasmon resonance experiments showed that the presence of Zn2⁺ but not other divalent cations altered the kinetics of the KIR–HLA-C association and dissociation, pointing to a direct role for Zn2⁺ in KIR multimerization (14). Zn2⁺-dependent dimerization of KIR may contribute to KIR clustering and to the inhibitory function of KIR. However, KIR dimers have not been detected at the surface of NK cells, even after chemical cross-linking (M. Peterson, unpublished). It is possible that KIR dimerization on NK cells is induced only transiently upon interaction with HLA-C. Additional KIR–KIR or KIR–class I interactions would be necessary to achieve clustering beyond KIR dimerization. A form of KIR–KIR clustering (handshake) was observed in a crystal of 2DL2 in the absence of the HLA-C ligand (41).

Crystal structures of KIR2D–HLA-C complexes have the potential to hold clues about KIR clustering upon binding to HLA-C on opposing target cells. The stoichiometry of 2DL2 bound to HLA-Cw3 was 1:1 in the crystal of the complex (42). The same 1:1 stoichiometry had been observed for 2DL1 bound to HLA-Cw4 in solution (43). A lattice array formed by a second contact of KIR to another HLA-C molecule was revealed by the packing in the 2DL2–HLA-Cw3 crystal (42). If this array occurs at the cell surface, it would result in polymerization of KIR on the NK cell and of HLA-C on the target cell, even though no direct KIR–KIR or HLA-C–HLA-C contacts are formed. Functional experiments with mutated KIR and HLA-C molecules are needed to test these models for KIR clustering.

SHP-1 binding to phospho-ITIM

The importance of a hydrophobic amino acid two residues upstream of the ITIM tyrosine for binding of SHP-1 (44) has been confirmed by in vitro binding and activation studies and by functional expression of mutated KIR (45, 46). The presence of an ITIM (V/IxxYxxL/V) in the cytoplasmic tail of receptors, such as gp49B, paired immunoglobulin-like receptor B (PIR-B), and PD-1, has been a reliable predictor of the inhibitory function of those receptors (47–50).

The weight of the evidence clearly favors a phospho-ITIM-
dependent recruitment of SHP-1 as a key step in the inhibitory signal. Recruitment of SHP-1 by KIR upon binding MHC class I on target cells has been detected (D. N. Burshtyn, unpublished). In that experimental context (Fig. 2A), tyrosine phosphorylation of SHP-1 was more easily detected than that of KIR (34). Overexpression of a catalytically inactive SHP-1 in NK cells prevented the inhibition mediated by KIR (44, 51). The inhibitory function of ITIM-containing Ly49A receptors in mouse NK cells was partially impaired in SHP-1-deficient moth-eaten mice, which express normal SHP-2, a SHP-1-related phosphatase (52). Complete impairment of an ITIM-mediated inhibition was observed in the DT40-cell line only after inactivation of both SHP-1 and SHP-2 genes (53). Phosphorylated KIR ITIM also bind SHP-2 (54, 55), which could compensate for the lack of SHP-1 in mutant cells. A mutant KIR in which only the first ITIM was preserved bound SHP-2 more efficiently than SHP-1 after cross-linking with activation receptors (55). This finding is relevant to the function of the KIR family members that have only the first ITIM, such as 2DL4 and 3DL2. Finally, chimeric KIR containing SHP-1 in place of its cytoplasmic tail inhibited NK cells, showing that SHP-1 is sufficient for the inhibitory signal (34, 56).

SHP-1 substrate selection during inhibition by KIR
Identification of the direct substrates of SHP-1 targeted for dephosphorylation during inhibition by KIR would clarify the mechanism of inhibition and help define pathways of NK activation. Although substrates that are directly dephosphorylated by SHP-1 have not been identified yet, it is not for a lack of candidates. Inhibition upon recognition of MHC class I on target cells (Fig. 2A) results in a selective reduction of tyrosine phosphorylation of a few proteins in NK cells (31, 32, 57). In one such study, total phosphorylated proteins were compared after mixing NK cells with sensitive or resistant target cells. Engagement of 3DL1 by HLA-Bw4-bearing targets resulted in a specific loss of proteins with an apparent molecular weight of 50, 135, and 158 (32). The phosphorylation state of specific proteins was monitored upon target cell mixing, and a decreased phosphorylation of syk and a Grb2-associated 36 kDa protein (presumably linker for activation of T cells (LAT)) were noted after mixing with resistant target cells (32, 57). A similar result was obtained with a γ/δ T-cell clone expressing inhibitory CD94/NKG2A whose engagement resulted in reduced tyrosine phosphorylation of an unknown 130 kDa protein and of the kinase ZAP70, but not the T-cell receptor ζ chain (33).

The system of redirected lysis of P815 cells (Fig. 2B) has been used extensively in functional studies of inhibitory receptors, but not for biochemical studies. In this system, activation through cross-linking of a chimeric CD16/syk receptor expressed in a T-cell line was inhibited by co-ligation of 2DL2/3, indicating that KIR can inhibit at the level, or downstream of, the tyrosine kinase syk (58).

The effect of KIR on the CD16 signal has been studied by mAb-mediated cross-linking (Fig. 2C). Inhibition through cross-linking of KIR and CD16 in NK-cell clones resulted in the loss of a prominent tyrosine phosphorylated band at 36 kDa as well as several bands in the 70–150 kDa range, in addition to a global reduction in protein tyrosine phosphorylation. Specifically, decreases in the CD16-induced tyrosine phosphorylation of the ζ chain, ZAP70, phospholipase Cγ1 (PLCγ1) and PLCγ2, and SLP-76 were noted (51, 58). A similar study evaluating the impact of CD94/NKG2A-mediated inhibition of CD16 signals reported a decrease in the tyrosine phosphorylation of syk, ζ chain, and shc (59). It is not known whether any of these molecules represent the direct substrate of SHP-1 or whether their reduced tyrosine phosphorylation is caused by dephosphorylation of an upstream substrate by SHP-1. Some of these molecules may not be necessary for NK cytotoxicity, as mice deficient in LAT, SLP-76, or Syk display normal NK function (60–62).

An approach that would directly identify SHP-1 substrates is the expression of SHP-1 trapping mutants (i.e. a mutant that binds but does not cleave phosphotyrosine) in NK cells. Such an approach was used in the macrophage cell line BAC1.2F5 and in primary bone marrow-derived macrophage cells to identify the ITIM-containing signal regulatory protein (SIRP) as a SHP-1 substrate (63). SIRP is a receptor that also binds to the SH2 domains of SHP-1. Similar trapping experiments have identified substrates in other cell types. In B cells, the B-cell linker protein (BLNK) was trapped following IgM cross-linking (64), and in mast cells two proteins of 25 and 30 kDa were detected following stimulation by antigen/IgE complexes (65). However, SHP-1 bound to these proteins in the absence of a specific inhibitory signal. To identify substrates of SHP-1 during KIR-mediated inhibition we have transfected an NK-cell line with a chimeric KIR receptor that contains SHP-1 in place of the KIR cytoplasmic tail. This chimeric receptor inhibits NK-cell function when it binds to HLA-C on target cells (34, 56). Expression of a KIR–SHP-1 chimeric receptor carrying a trapping mutation in the catalytic domain of SHP-1 is currently in use to identify substrates of SHP-1 that are involved in NK activation signals.

Further information on potential SHP-1 substrates can be derived from the crystal structure of the SHP-1 catalytic do-
Fig. 3. Inhibition of 2B4 tyrosine phosphorylation. A. Purified human NK cells were preincubated with inhibitors as described in A, mixed with the target 221-Cw7 or 221-Cw3 at an E/T ratio of 2 and incubated at 37°C for the indicated amount of time. Note that the HLA-E-negative 221-Cw7 cells were sensitive to killing by the NK cells whereas the HLA-E-expressing 221-Cw3 cells were protected through the engagement of the inhibitory receptor CD94/NKG2A on NK cells (34). Subsequently, cells were lysed and immunoprecipitated with a control IgG1 (C) or anti-2B4. Immunoprecipitates were analyzed as described in A.

KIR inhibits tyrosine phosphorylation of the activating receptor 2B4 (CD244)

During contact with sensitive target cells, NK cells are activated by the engagement of various surface receptors (67). Although this activation process is poorly understood, it is clear that inhibitory KIR can block several different signaling pathways. To explore how KIR may be interfering with the activation pathway, we studied the influence of KIR on 2B4-mediated NK activation. 2B4 contains four tyrosine motifs (TxYxxI/V) in its cytoplasmic tail, similar to those found in CD84, Ly9, and the T-cell stimulatory receptor CD150. The 2B4 signal can synergize with other ITAM-based activation receptors, such as Nkp46 and CD16 (68). Tyrosine-phosphorylated 2B4 binds the small SH2 domain-containing molecule SAP (also called SH2D1A) or the phosphatase SHP-2, but not both at the same time (69). Co-immunoprecipitation suggests a constitutive association of 2B4 with the scaffold protein LAT (70). Patients with the X-linked lymphoproliferative disease who lack a functional SAP molecule have defective NK activation through the 2B4 receptor, implying an essential role of SAP in 2B4 signaling (71–74). However, further insight into the mechanism of NK activation by 2B4 is still missing. Tyrosine phosphorylation of 2B4 can be directly induced by antibody cross-linking, demonstrating that this phosphorylation does not depend on other activating receptors (34). Similarly, in cell mixing experiments, engagement of 2B4 by its ligand CD48 expressed on sensitive target cells resulted in rapid 2B4 phosphorylation. In contrast, 2B4 phosphorylation was inhibited by the binding of either KIR or CD94/NKG2A to their respective MHC class I ligands on target cells (Fig. 3) (34). Therefore, inhibitory receptors can interfere with a step as proximal as phosphorylation of an activation receptor.

Although it is possible that SHP-1 dephosphorylated 2B4...
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Fig. 4. Models of inhibition by KIR. A. KIR inhibits through the SHP-1-mediated dephosphorylation of multiple targets, such as 2B4, other activation receptors (X), and downstream signaling molecules (Y). B. KIR targets a central signaling component (Z) for dephosphorylation that is downstream of 2B4 and other activating receptors (X), and is essential for full 2B4 phosphorylation, target cell adhesion through LFA-1, and NK activation.

directly, even signals that are upstream of 2B4 phosphorylation may be targets for SHP-1 during inhibition. 2B4 phosphorylation depends on the activity of an src-family kinase: overexpression of the src kinase fyn induces 2B4 phosphorylation, and pervanadate-induced 2B4 phosphorylation is greatly reduced in 2B4-transfected JCam cells that lack the src kinase lck (71, 75). Similarly, we found that 2B4 phosphorylation induced by cross-linking with antibodies in normal NK cells was blocked by the src kinase inhibitor PP1 (Fig. 3A). In contrast, whereas the syk inhibitor piceatannol did not inhibit 2B4 phosphorylation induced by cross-linking with antibodies, it partially blocked 2B4 phosphorylation induced by target cell mixing (Fig. 3). This difference underscores the importance of interpreting results in the context of the experimental system. 2B4 phosphorylation after cross-linking with antibody may depend only on src kinases, such that co-cross-linked KIR may inhibit by direct dephosphorylation of 2B4 or the relevant src kinase (Fig. 4A). In contrast, phosphorylation of 2B4 during mixing with target cells requires more complex signals, making it possible that KIR inhibits by targeting another signaling component of NK-cell activation for dephosphorylation by SHP-1 (Fig. 4B).

KIR inhibits NK-cell adhesion to target cells

Target cell killing is the outcome of multiple steps in the NK-cell activation process, with formation of a tight interface between NK and target cells occurring early, and with polarized degranulation occurring at a late step. The β2 integrin leukocyte function-associated antigen (LFA)-1 (CD11a/CD18) plays an important role in adhesion of NK cells by binding to intercellular adhesion molecule-1 (ICAM-1) on target cells. Tight adhesion through LFA-1 is itself dependent on inside-out signals that increase the affinity of LFA-1 and induce cytoskeletal rearrangements (76). Anti-CD11a antibodies blocked the formation of conjugates between the NK-cell line YTS and target cells, underscoring the central role of LFA-1 in the adhesion process (56). The src kinase inhibitors PP1 and herbimycin A reduced conjugate formation and blocked target cell lysis, suggesting a role for specific signals in the tight adhesion between YTS and target cells. The dependence of adhesion on intracellular signals provides potential targets for inhibition by KIR. YTS–target cell adhesion was interrupted rapidly by recognition of MHC class I on target cells by inhibitory KIR (56). The activity of SHP-1 is sufficient for this inhibition, as the same effect was observed using NK cells expressing a KIR/SHP-1 chimera in which the cytoplasmic tail of KIR was replaced with SHP-1. Therefore, KIR can interfere through the activity of SHP-1 with signals that lead to tight adhesion to target cells. The block in adhesion may contribute to the protection of normal cells from NK killing and may allow NK cells to scan potential targets more efficiently by accelerating the release from cells expressing the right MHC class I ligand.

Conclusions

Two models can explain the inhibitory effect of KIR on different elements of the NK activation pathway. Activation of SHP-1 by KIR could result in the dephosphorylation of multiple targets, including candidates such as 2B4 and signaling components of the inside-out signal for LFA-1 (Fig. 4A). Alternatively, KIR may target only one or very few central signaling components that are essential for adhesion to target cells and NK-cell cytotoxicity (Fig. 4B). The fact that different
tyrosine kinases contribute to 2B4 phosphorylation during target cell contact may reflect a dependence on a central signaling component that controls adhesion and 2B4 phosphorylation. Identification of SHP-1 substrates using a trapping mutant of SHP-1 during inhibition by KIR will put these models to a direct test.

Progress in unraveling the KIR inhibitory pathway is hindered by an incomplete picture of NK activation signals. To dissect the contribution of signals from individual receptors to the various steps in the activation pathway, we are developing systems to reconstitute individual steps such as recognition of ligands by adhesion molecules and activating receptors. Beads coated with either ligands or mAbs to receptors have been used successfully to elicit polarized responses by T cells (77). However, cell-based assays are preferable; for instance, beads coated with chimeric 2DL1-Fc failed to induce clustering of HLA-Cw4 on target cells, indicating that lateral mobility of KIR within the membrane is necessary for clustering (37). Other systems such as planar lipid membranes or transfected cell lines that do not express ligands for NK receptors will approximate the NK–target cell interaction with greater fidelity. Expression of ligands for NK-activating receptors and co-expression of MHC class I molecules in such systems should extend our knowledge of the mechanisms underlying NK activation and the steps in the activation process that are directly inhibited by KIR.

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