Functional Role for Syk Tyrosine Kinase in Natural Killer Cell–mediated Natural Cytotoxicity

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Summary

Natural killer (NK) cells are named based on their natural cytotoxic activity against a variety of target cells. However, the mechanisms by which sensitive targets activate killing have been difficult to study due to the lack of a prototypic NK cell triggering receptor. Pharmacologic evidence has implicated protein tyrosine kinases (PTKs) in natural killing; however, Lck-deficient, Fyn-deficient, and ZAP-70-deficient mice do not exhibit defects in natural killing despite demonstrable defects in T cell function. This discrepancy implies the involvement of other tyrosine kinases. Here, using combined biochemical, pharmacologic, and genetic approaches, we demonstrate a central role for the PTK Syk in natural cytotoxicity. Biochemical analyses indicate that Syk is tyrosine phosphorylated after stimulation with a panel of NK-sensitive target cells. Pharmacologic exposure to piceatannol, a known Syk family kinase inhibitor, inhibits natural cytotoxicity. In addition, gene transfer of dominant-negative forms of Syk to NK cells inhibits natural cytotoxicity. Furthermore, sensitive targets that are rendered NK-resistant by major histocompatibility complex (MHC) class I transfection no longer activate Syk. These data suggest that Syk activation is an early and requisite signaling event in the development of natural cytotoxicity directed against a variety of cellular targets.

The NK cell is a type of lymphocyte that is able to mediate natural cytotoxicity against a variety of tumor cells, virus-infected cells, and hematopoietic targets (1). However, little is known regarding the mechanisms by which these targets trigger natural cytotoxicity. In addition to natural cytotoxicity, the NK cell can mediate antibody-dependent cell-mediated cytotoxicity (ADCC) through its FcR for IgG, FcγRIII. In contrast to natural cytotoxicity, ADCC is well defined with respect to the receptor–ligand interaction as well as intracellular second messenger involvement (for review see reference 2). In spite of this contrast, between the well-defined FcR-dependent and the poorly defined natural cytotoxic mechanisms, both killing mechanisms can be inhibited by MHC-recognizing killer cell inhibitory receptors (KIR). Since KIR inhibition seems to target signaling pathways initiated by immunoreceptor tyrosine-based activation motif (ITAM)-containing receptor complexes (e.g., FcγR III, TCR, and FcεRI; references 3–9), the ability of both FcR-dependent and natural cytotoxicity to be inhibited by KIR suggests that there may be shared signaling elements in the pathways used during the two alternative routes of initiating NK cell-mediated cytotoxicity.

Evidence using the PTK inhibitors herbimycin A and genistein demonstrates that PTK activation is necessary for both FcR-mediated and natural cytotoxicity (10–14). However, the available genetic evidence from Lck- and Fyn-deficient mice suggests that neither of these specific Src family tyrosine kinases is necessary for NK cell-mediated cytotoxicity (15, 16). This raises the possibility that another Src family PTK (e.g., Lyn) could subserve this role. Alternatively, the receptors initiating NK cell-mediated killing could use a different kind of PTK that might function in a Src family kinase-independent manner. The NK family member ZAP-70, which is expressed in NK cells, is unlikely to be this PTK because ZAP-70 appears to require activation by a Src family PTK (17–20); and because NK cells from humans and mice lacking ZAP-70 can mediate normal natural cytotoxicity and ADCC (21–23). In contrast, Syk itself could perform this role. Syk is expressed in

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1Abbreviations used in this paper: ADCC, antibody-dependent cell-mediated cytotoxicity; ITAM, immunoreceptor tyrosine-based activation motif; KAR, killer cell activating receptor; KARAP, KAR-associated protein; KIR, killer cell inhibitory receptor; PTK, protein tyrosine kinase.

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In the absence of Src family kinases, Syk is able to function. Although chimeric receptors containing the intracellular portion of ZAP-70 require cross-linking with Src family kinases such as Lck or Fyn to mediate cytotoxicity, chimeric receptors containing Syk can initiate killing in the absence of Src family PTK cosimulation. In addition, Syk, but not ZAP-70, can induce the tyrosine phosphorylation of ITAM-containing receptor subunits in an Src family PTK-independent manner. To test the hypothesis that the Syk tyrosine kinase is functionally involved in natural cytotoxicity, we first stimulated NK cells with a panel of sensitive targets and then characterized their intracellular signaling events. Although stimulation of NK cells with sensitive targets is known to induce increases in intracellular free Ca$^{2+}$ and inostol phosphate release (26–30), more proximal specific signaling events have not been elucidated. In this study, we describe a rapid and reversible increase in the tyrosine phosphorylation of NK cell–derived Syk after target cell stimulation. In addition, either pharmacologic inhibition of Syk kinase activity or expression of dominant-negative, kinase-inactive Syk in NK cells inhibits natural killing. Finally, tumor cells that are made resistant to NK cell–mediated cytotoxicity by MHC class I transfection no longer activate Syk. Together, these data emphasize the central contribution of the Syk tyrosine kinase to the generation of natural cytotoxicity.

**Materials and Methods**

Cells, Chemicals, and Abs. The P815 cell line (murine mastocytoma) was obtained from American Type Culture Collection (Rockville, MD). The HLA class I–deficient C1R cell line (B-LCL) and its HLA–transfected derivatives were supplied by Peter Cresswell (Yale University, New Haven, CT). The HLA class I–deficient cell line 721.221 (B-LCL) was provided by Peter Parham (Stanford University, Palo Alto, CA). Human PBL were isolated from defibrinated blood by Ficoll-Hypaque density centrifugation. Human CD16$^+$ NK cell lines were isolated and characterized as previously described (29). All chemicals, unless otherwise noted, were obtained from Sigma Chemical Co. (St. Louis, MO). The 3G8 hybridoma (anti-FcγRIII) was provided by Bice Perussia (Thomas Jefferson University, Philadelphia, PA; reference 31) and 3G8 mAb was purified by affinity chromatography over protein A agarose. The generation and characterization of rabbit antiserum specific for ZAP-70 have been previously described (32). The 4D10 mAb specific for Syk was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The antiphosphotyrosine mAb 4G10 was purchased from Upstate Biotechnology (Lake Placid, NY).

Cytotoxicity Assays. The $^{51}$Cr-release assays measuring direct NK cell–mediated cytotoxicity were performed as previously described (29). For experiments evaluating the effects of piceatannol (Boehringer Mannheim, Indianapolis, IN), cloned human NK cells were pretreated with the indicated concentration of the drug for 15 min at 37°C. Lytic units were calculated based on 20% cytotoxicity (33).

Vladimira Vinueza. ZAP-70K cDNA was excised from pcDNA3. KD.myc.Zap70 (provided by Lawrence E. Samelson, National Institutes of Health, Bethesda, MD; reference 34) with BamHI/NotI, and blunt-ended with Klenow. The blunt-ended ZAP-70K fragment was inserted into the Smal cloning site of the vector pSC11 and introduced into WR strain vaccinia virus via homologous recombination. Recombinant vaccinia viruses encoding wild-type Syk, wild-type ZAP-70, SykT, and SykK as well as the pSC-65 vector were provided by Jean-Pierre Kinet and Andrew M. Scharenberg (Harvard Medical School, Boston, MA; references 35, 36). For infections, NK cells (2 × 10$^6$ cells/ml) were incubated in serum-free RPMI 1640 for 1 h at 37°C at a multiplicity of infection of 20. Cells were then incubated for the remainder of the indicated infection time at 10$^6$ cells/ml in RPMI 1640 supplemented with 10% bovine calf serum.

Whole Cell Lysates, Cell Stimulation, Immunoprecipitation, and Immunoblot Analysis. For whole cell lysates, 1.5 × 10$^6$ NK cells were lysed in buffer containing 10 mM Tris (pH 7.4), 50 mM NaCl, 5 mM EDTA, 50 mM Naf, 30 mM Na$_2$PO$_4$, and 1% Triton X-100 (pH 7.4). Protein levels were determined using the BCA Protein Assay (Pierce, Rockford, IL) and equal amounts of protein were resolved by SDS-PAGE. For cell stimulation, NK cells were resuspended at 10$^5$ cells/ml, and target cells were resuspended at 5 × 10$^6$ cells/ml in RPMI 1640 supplemented with 0.5% BSA. 100 μl of NK cells were mixed with 100 μl of target cells (effector:target ratio of 2:1), pelleted at 5,000 rpm for 5 s, and then incubated at 37°C for the indicated time. For anti-FcR stimulation, NK cells were incubated at 4°C for 3 min with anti-FcγR mAb (3G8; 10 μg/ml). Washed cells were then mixed with goat anti–mouse IgG F(ab')2 fragments (Organon Teknika-Cappel, Durham, NC), rapidly pelleted, and incubated for 1 min at 37°C. After stimulation, cells were lysed in buffer containing 20 mM Tris-HCl, 40 mM NaCl, 5 mM EDTA, 50 mM Naf, 30 mM Na$_2$PO$_4$, 0.1% BSA, 1 mM NafVO$_4$, 1 mM PMSF, 5 μg/ml aprotinin, 10 μg/ml leupeptin, and 1% Triton X-100 (pH 7.4). Samples were centrifuged at 15,000 g for 5 min to remove insoluble material. Supernatants were subjected to immunoprecipitation with either rabbit antiserum specific for ZAP-70 bound to protein A–Sepharose or goat anti–mouse IgG bound to protein A–Sepharose. Immunoprecipitates were washed and bound proteins were eluted with 50 μl of SDS-sample buffer. Proteins resolved by SDS-PAGE were electrophoretically transferred to Immobilon-P membranes (Millipore, Bedford, MA).

Tyrosine phosphorylated proteins and Syk expression were detected with the 4G10 and 4D10 mAbs, respectively, followed by sheep anti–mouse IgG coupled to horseradish peroxidase (Amerham International, Buckinghamshire, England). ZAP-70 expression was analyzed with ZAP-70–specific rabbit antiserum and detected with protein A–horseradish peroxidase and the ECL detection system from Amerham.

$^{32}$P-labeling and Phosphoamino Acid Analysis. NK cells were washed with phosphate-free RPMI 1640 medium supplemented with 4% bovine calf serum and 1% l-glutamine. Cells were labeled with 0.5 μCi of [32P]orthophosphate/ml for 3 h at 37°C at 1.5 × 10$^7$ cells/ml in phosphate-free medium, washed twice, and then resuspended at 10$^6$ cells/ml, and 100 μl of NK K cells were used for stimulation. Target cells were also washed with phosphate-free medium (not containing [32P]orthophosphate) before use for cell stimulation.

For phosphoamino acid analysis, portions of the membrane corresponding to Syk were subjected to autoradiographs and cut and boiled in 6 N HCl for 1 h at 110°C to elute the protein. Membrane fragments were rinsed twice with water and rinses were combined with eluate. Eluate plus rinse was dried in a speed-vac and resuspended in water/ethanol/methanol (10:5:1) before spotting.
on thin-layer chromatography (TLC) plates. Two cycles were run in solvent ethanol/ammonium hydroxide/water (105:42:6) with phosphoamino acid standards. TLC plates were sprayed with 0.2% ninhydrin in ethanol before exposure to film.

**Results**

NK-sensitive targets induce tyrosine phosphorylation of NK cell Syk. Although PTK activation is an early and requisite event in the generation of natural cytotoxicity (10, 11), genetic evidence suggests that the PTKs Lck, Fyn, and ZAP-70 are not required (15, 16, 21–23). These observations prompted us to evaluate whether the tyrosine kinase Syk is important for natural cytotoxicity. We first determined whether Syk was tyrosine phosphorylated after stimulation of NK cells with sensitive targets since an increase in Syk tyrosine phosphorylation correlates with increased Syk activation. Human NK cell clones were incubated either with the MHC class I–deficient B lymphoblastoid targets C1R or 721.221 or with the prototypic human NK cell target K562. After various times of incubation, cells were lysed and Syk was immunoprecipitated. We observed an increase in Syk tyrosine phosphorylation after NK cells were stimulated with each of the panel of targets (Fig. 1, A–C). This increase in Syk tyrosine phosphorylation was a relatively early event, peaking at 5 min and declining to baseline by 60 min (Fig. 2). Since both Syk family members Syk and ZAP-70 are expressed in NK cells and activated downstream of the FcR (24, 32, 37), we questioned whether NK cell–derived ZAP-70 was also tyrosine phosphorylated after incubation with sensitive targets. ZAP-70 immunoprecipitates from target cell–stimulated lysates revealed no increase in the tyrosine phosphorylation of ZAP-70 after target cell contact (Fig. 1, A–C). This was not a defect in the ability of ZAP-70 to be phosphorylated in these NK cells since cross-linking with FcR-specific antibodies on the same cells results in an increase in ZAP-70 tyrosine phosphorylation (Fig. 1, A–C). Thus, it appears that Syk and not ZAP-70 is activated when this panel of targets is used to stimulate NK cells. Consistent with the observations that both Syk and ZAP-70 are tyrosine phosphorylated after FcR ligation (24, 32, 37), stimulation of NK cells with the 3G8 hybridoma, which expresses membrane-bound antibody to the FcR, resulted in an increase in both Syk and ZAP-70 tyrosine phosphorylation (Fig. 3). These data suggest that while FcR cross-linking can stimulate the tyrosine phosphorylation of Syk and ZAP-70, stimulation of NK cells with this panel of sensitive targets results in the phosphorylation of Syk without detectable ZAP-70 phosphorylation.

Although Syk activation has not been described in tumor cells undergoing cell death, we could not formally exclude the possibility that the endogenous Syk in the above panel of targets was undergoing tyrosine phosphorylation. To ensure that the observed increase in tyrosine phosphorylation was NK cell–derived Syk, NK cell clones were labeled with \(^{32}\)P orthophosphate before cellular target stimulation. Indeed, Syk immunoprecipitates showed an increase in \(^{32}\)P labeling.
Kinase-inactive Syk Expression Inhibits Killing.

To express wild-type or kinase-inactive forms of Syk family PTK in the development of natural cytotoxicity, we took a genetic approach to further define a role for Syk family PTK in natural cytotoxicity, we used the SykT mutant, which has recently been shown to act as a dominant-negative in mast cells (35), is truncated at residue 395 so as to lack the kinase domain but contain the SH2 domains. The kinase-inactive SykK mutant has a point mutation at residue 395 (K to R) that is within the ATP-binding region of the kinase domain. Kinase inactive ZAP-70 (ZAP-70K) also has a K to R point mutation at residue 395 within the kinase domain. After a 4 h infection with virus containing the coding sequences either for wild-type Syk family PTK, mutant Syk family PTK, or the control vector, NK cell function was assessed in a 51Cr-release assay. Overexpression of wild-type Syk with vaccinia enhanced natural killing whereas wild-type ZAP-70 overexpression did not (Fig. 6). The kinase-inactive mutants of Syk (SykT, SykK) but not ZAP-70 (ZAP-70K) acted as dominant-negatives to inhibit natural killing (Fig. 6). Although the latter observation is consistent with the notion that ZAP-70 is not required for natural cytotoxicity, one cannot exclude the possibility that the inability of ZAP-70K to inhibit killing is due to insufficient expression of the transfected gene product. Therefore, we took a genetic approach to directly analyze this issue. Specifically, we derived NK cell clones from a patient with ZAP-70 deficiency (Fig. 7 A) and found that these clones mediated wild-type levels of natural killing (Fig. 7 B) as previously reported (21, 22). Taken together, the above results suggest a required role for Syk, but not ZAP-70, in natural killing. It should be emphasized that although our data indicate that ZAP-70 activity is not neces-

Figure 4. 32P incorporation into NK cell-derived Syk. 107 32P-labeled NK cells were mixed with 5 × 106 cells of the indicated target (K562 or C1R), pelleted, and incubated at 37°C for the indicated times. Syk immunoprecipitates were resolved by SDS-PAGE, transferred to membrane, and exposed to x-ray film (Syk) followed by probing with antiphosphotyrosine mAb (P-Tyr blot). cpm from phosphoamino acid analysis were: NK K562 — 5′: 2.3-fold increase in [32P]Tyr, 2-fold increase in [32P]Ser; NK K562 — 10′: 1.5-fold increase in [32P]Tyr, 1.5-fold increase in [32P]Ser; NK C1R — 5′: 2.4-fold increase in [32P]Tyr, 1.6-fold increase in [32P]Ser; NK C1R — 10′: 5.4-fold increase in [32P]Tyr, 2.5-fold increase in [32P]Ser.

Figure 5. Piceatannol pretreatment inhibits NK cell-mediated cytotoxicity. NK cells were treated for 15 min at 37°C with the indicated concentration of piceatannol, washed, and then incubated for 4 h with either 51Cr-labeled K562 cells or 51Cr-labeled P815 cells coated with 0.15 μg/ml of the anti-FcR mAb 3G8. Data are expressed as percentage of inhibition of lytic U/106 cells calculated from NK cells incubated in vehicle alone.

Figure 6. Kinase-inactive Syk inhibits the generation of natural cytotoxicity. NK cells were infected for 4 h either with vector control vaccinia (pSC-65) or recombinant vaccinia encoding wild-type Syk, wild-type ZAP-70, truncated Syk (SykT), kinase-inactive Syk (SykK), or kinase-inactive ZAP-70 (ZAP-70K). Infected cells were incubated for 4 h with 51Cr-labeled 721.221 cells. Data are expressed as lytic U/106 cells ± one standard deviation.
ZAP-70 is not required for natural cytotoxicity. (A) Whole cell lysates from normal and ZAP-70-deficient NK cells were resolved by SDS-PAGE, transferred to membrane, and probed with anti-ZAP-70 antisera (ZAP-70) or anti-Syk mAb (Syk). (B) Normal and ZAP-70-deficient NK cells were incubated for 4 h with ^3^H-labeled 721.221 cells.

**Figure 7.** ZAP-70 is not required for natural cytotoxicity. (A) Whole cell lysates from normal and ZAP-70-deficient NK cells were resolved by SDS-PAGE, transferred to membrane, and probed with anti-ZAP-70 antisera (ZAP-70) or anti-Syk mAb (Syk). (B) Normal and ZAP-70-deficient NK cells were incubated for 4 h with ^3^H-labeled 721.221 cells.

Discussion

NK cells exhibit unprimed, natural cytotoxicity against many different targets (1). Here we examine the mechanisms by which NK cells generate cytotoxicity against a panel of tumor targets. We demonstrate that the PTK Syk plays a central role in NK cell-mediated cytotoxicity.

Mechanisms of natural cytotoxicity have been difficult to study due to the lack of defined triggering receptors. A number of candidate receptors have been described which can induce certain aspects of NK cell activation and/or cytotoxicity. Among these receptors are adhesion molecules such as the β1- and β2-integrins (40–44), and other receptors such as CD2 (45), CD69 (46), Lag-3 (47), DNAM-1 (48), KAR (4, 49–57), NK-TR (58), and NKR-P1 (59–61). Antibodies to some of these receptors can enhance the killing of FcR-bearing resistant targets (“reverse ADCC”) as well as stimulating the release of inositol phosphates and intracellular free Ca^{2+}. However, proving the direct involvement of such receptors in the natural killing of specific tumor targets has often been problematic. Given the number of NK cell receptors that are candidate triggering receptors and the broad range of sensitive target cells, the receptor–ligand interactions involved may be relatively specific for each effector–target combination. In addition to the mere presence or absence of triggering receptors on NK cells or triggering ligands on target cells, the distribution of these proteins in the cell membrane may be critical. Helander et al. (44) propose the β2-integrin LFA-1 as a receptor able to trigger natural cytotoxicity by recognizing its redistributed ligand ICAM (intracellular adhesion molecule)-2 on diseased versus normal cells. Their results sug-
gest that when ICAM-2 is concentrated on the target cell membrane, LFA-1 can participate in triggering cytotoxicity. Given the potential heterogeneity in triggering receptors required for natural cytotoxicity and the possibility that ligand distribution on the target cell may be involved, we used a panel of NK-sensitive tumor targets to stimulate NK cell clones rather than isolating receptor-ligand interactions to examine the molecular mechanisms involved in natural killing. Indeed, our data suggest that for this panel of targets, the Syk tyrosine kinase plays a common regulatory role in the generation of natural cytotoxicity.

The molecular mechanisms by which Syk is activated during natural cytotoxicity are unknown. In contrast to the poorly defined receptor-ligand interactions involved in natural cytotoxicity, FcR-initiated killing has a well-defined receptor and signaling pathway. FcγRIII consists of a ligand-binding α chain noncovalently associated with homo- or heterodimers of the CD3-ζ and FcεRIγ chains (62–65). The ζ and γ chains contain ITAMs that are thought to serve as substrates for Src family PTK (for reviews see references 66 and 67). Once these ITAMs are phosphorylated, the Syk family members can bind to the ITAM via their SH2 domains and activate downstream pathways. FcγRIII can induce tyrosine phosphorylation and activation of both Syk and ZAP-70 (24, 32, 37) but, similar to natural cytotoxicity, expression of dominant-negative, kinase-inactive forms of Syk but not ZAP-70 inhibit FcR-initiated killing (data not shown). This suggests that although Syk and ZAP-70 are activated after FcR cross-linking, ZAP-70 is not required for FcR-mediated cytotoxicity. These results are consistent with results from ZAP-70-deficient humans and mice showing no defects in natural cytotoxicity or ADCC (21–23).

Since our data suggest that Syk is a common signaling element used by FcR-initiated killing and natural cytotoxicity and since Syk family kinases are thought to dock to ITAM-containing molecules, this raises the possibility that ITAM-containing receptor complexes might be involved in natural cytotoxicity. The known ITAM-containing subunits expressed in NK cells, ζ and γ, do not appear to subserve this role in initiating natural cytotoxicity since NK cells from animals lacking these genes mediate normal natural killing (68–70). Killer cell activating receptors (KARs), variants of the KIRs that lack the cytoplasmic inhibitory motif required to bind the phosphatase SHP-1, have the capability to initiate NK cell-mediated killing (4, 49–57). Recent data suggest that KARs coprecipitate with molecular weights of ~12–16 kD (KAR-associated proteins or KARAPs) that are not ζ or γ (71). Although the molecular identities of these molecules are unknown, two-dimensional gel analysis suggests that, similar to ζ and γ, they may be expressed as disulfide-linked dimers. Expression of KARAPs appears to correlate with the ability of KARs to activate granule release, since cross-linking KAR on NK cells, which express KARAP, can trigger cytotoxicity while cross-linking KAR-transfected rat basophilic leukemia cells, which do not express detectable KARAP, does not induce granule release (71). Molecular cloning of the KARAP protein will be needed to determine if these are indeed additional ITAM-containing subunits that are shared by other triggering-receptor complexes.

Our data clearly suggest that Syk and ZAP-70 are not functionally redundant in the generation of natural cytotoxicity. This observation is consistent with reports demonstrating different activation requirements for each of the Syk family members. In vitro studies using peptides containing phosphorylated ITAMs from the FcεRIγ chain suggest that these peptides can activate Syk kinase activity (72, 73), whereas ZAP-70 kinase activity is only activated upon binding a dimeric form of phosphorylated ζ (74) rather than peptide fragments (75, 76). In vivo studies in COS cells suggest that ZAP-70 is only phosphorylated upon the addition of Lck or Fyn (17), whereas Syk is phosphorylated when transfected alone (77, 78). The intrinsic enzymatic activities of Syk and ZAP-70 also differ when expressed in COS cells (79). Syk autophosphorylation and catalytic activity toward the exogenous substrate erythrocyte band 3 was ~100-fold greater than that of ZAP-70. Further studies in COS cells expressing chimeric receptors which cytoplasmically express FcεRIγ chain coexpressed with Syk or ZAP-70 showed that the chimeric γ plus Syk led to γ phosphorylation and Syk kinase activity while γ plusZAP-70 induced neither γ phosphorylation nor ZAP-70 activation (20).

The final outcome of an NK cell interacting with a target cell is determined by the balance of positive and negative signals transmitted. Positive signals generated during natural cytotoxicity include activation of PTKs and increases in inositol phosphate and intracellular free Ca²⁺ release. Negative signals are derived, at least in part, from the protein-tyrosine phosphatase (PTPase) SHP-1 that is associated with MHC-recognizing KIR (5, 8, 80–82). Although it appears the overall balance of positive and negative signals generated determines the outcome of an NK cell interacting with a target cell, our data suggest that the two pathways may directly interact. KIR may preempt the initiation of a positive signal by acting on the activating receptor complex or its most proximal signaling molecules. Since Syk activation is central to the generation of both natural cytotoxicity and ADCC, SHP-1-mediated dephosphorylation of Syk could inhibit cellular activation. Alternatively, SHP-1 may act by inactivating a kinase that may phosphorylate and activate Syk. Recent data suggest that Syk may be activated by an Src kinase-initiated activation loop in which, once a small amount of Syk is phosphorylated, it can activate other Syk molecules to allow rapid Syk activation (36). Another potential SHP-1 target is the ITAM-containing receptor protein that upon phosphorylation docks Syk family molecules to the receptor complex. SHP-1 targeting any of these activation steps could inhibit the Syk tyrosine kinase by either preventing the association of Syk with the receptor complex or by preventing its full activation. Valiante et al. (83) have suggested that KIR-associated SHP-1 inhibits NK cell-mediated natural killing by dephosphorylating such downstream targets as the phospholipase C (PLC)-γ-adaptor protein pp36, thereby pre-
venting PLC-γ association and activation. Our results here, together with previous data from our lab (4,5), suggest that inhibition may occur at a more proximal step in activation. If inhibition of Syk activation, a proximal step in the generation of natural cytoxicity and ADCC, is the target for KIR inhibition, then this supports a role for Syk as a common second messenger in use during many forms of cytoxicity.

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