Induction of Protein Tyrosine Phosphorylation in Human Natural Killer Cells by Triggering Via \( \alpha_4\beta_1 \) or \( \alpha_5\beta_1 \) Integrins

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Recent studies have shown that cell-surface integrins expressed on platelets, fibroblasts, or carcinoma cell lines serve not only as adhesion receptors that connect the extracellular matrix to the cytoskeleton, but also as signal-transducing molecules involved in altering cellular patterns of tyrosine phosphorylation. In this present report we provide evidence that adhesion of freshly purified human natural killer (NK) cells to fibronectin (FN) induces tyrosine phosphorylation of intracellular proteins of approximate molecular mass of 60, 70, and 120 kD. Increases in phosphorylation induced by NK cell binding to immobilized FN were partially blocked by EILDV-(CS-1) or RGD-containing peptides, which compete specifically for a distinct binding site for either \( \alpha_4\beta_1 \), or \( \alpha_5\beta_1 \) integrins, respectively, within the FN molecule. The presence of either one of the inhibitory peptides alone inhibited tyrosine phosphorylation primarily during short-term (30 minutes) and, to a lesser extent, during long-term (2 to 3 hours) periods of adhesion. These observations indicate that triggering either via \( \alpha_4\beta_1 \), or \( \alpha_5\beta_1 \), integrins, which are constitutively expressed on NK cells, induces protein tyrosine phosphorylation. Moreover, FN fragments of 40 or 120 kD, known to contain the binding sites for \( \alpha_4\beta_1 \), or \( \alpha_5\beta_1 \) integrins, respectively, used as immobilized substrates for NK cell adhesion, were able to initiate tyrosine kinase activity. The induced tyrosine phosphorylation was observed mainly on intracellular proteins of greater than 50 kD molecular weight. We have identified a 70-kD tyrosine phosphoprotein as paxillin, a cytoskeletal-associated tyrosine kinase substrate previously identified in fibroblasts and shown to localize to focal adhesions. Thus, interaction of NK cells with immobilized extracellular matrix glycoproteins required for migration and extravasation of these cells involves activation of intracellular protein tyrosine kinases and tyrosine phosphorylation of cytoskeleton-associated protein, paxillin, which may play a role in signaling between \( \beta_1 \) integrins and the underlying cytoskeleton.

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THE INTERACTION of cells with extracellular matrix (ECM) proteins has an important effect on a variety of cellular functions such as growth, migration, and differentiation. Many of the functional effects of ECM proteins are mediated by their binding to integrins. Integrins are cell-surface receptor proteins for a variety of ligands, including the ECM proteins, fibronectin, laminin, collagen, and vitronectin. All known integrins are heterodimers of non-covalently associated \( \alpha \) and \( \beta \) subunits. Both subunits of integrins are transmembrane glycoproteins that contain a large extracellular domain, a single transmembrane segment, and a short cytoplasmic domain. The \( \alpha \beta \) associations determine the ligand-binding specificities of the integrin heterodimers for various ECM proteins, and might transduce different signals from the ECM to the cell interior. Although integrins do not have characteristics of signaling receptors, such as kinase or phosphatase domains or a sequence suitable for interaction with G protein, evidence from several studies suggests that integrins can transmit biochemical signals into the cell. Thus, interactions of integrins with their protein ligands have been shown to modulate pH, activation of T lymphocytes, B lymphocytes and neutrophils, tumor-genicity, and gene expression in fibroblasts and monocytes.

Recent studies have shown that fresh human natural killer (NK) cells constitutively express \( \alpha_4\beta_1 \) and \( \alpha_5\beta_1 \) integrins, which mediate their binding to FN, and which binds laminin. Other \( \beta_1 \) integrins, including \( \alpha_5\beta_1 \), \( \alpha_6\beta_1 \), and \( \alpha_9\beta_1 \), are not expressed on fresh NK cells, but are induced after cellular activation. NK cells are known to play an important role in the host defense against viruses and tumor invasion. To mediate effector and immunoregulatory functions in tissues, NK cells have to migrate to the sites of infection or tumor growth. Migration across the vascular endothelium to gain access to tissue sites requires recognition of ECM molecules by NK cells, and thus, expression of integrins on these cells plays an important role in their interactions with the ECM. It is also possible that \( \beta_1 \) integrins expressed on NK cells are involved in signal transduction. However, transmembrane signaling by \( \beta_1 \) integrins in NK cells has not yet been studied. In this present study, we examine the possibility that crosslinking of \( \beta_1 \) integrins on fresh human NK cells by its ligand FN or fragments of FN induces activation of the tyrosine kinase pathway. We report that binding of NK cells to immobilized FN leads to tyrosine phosphorylation of several intracellular proteins and is mediated by either \( \alpha_4\beta_1 \) or \( \alpha_5\beta_1 \) integrins. We identified a focal adhesion protein, paxillin, that becomes tyrosine phosphorylated after interaction of NK cells with immobilized FN. By using peptides, which compete specifically for a distinct binding site for each integrin within the FN molecule, or fragments of FN known to contain the binding sites for either \( \alpha_4\beta_1 \) or \( \alpha_5\beta_1 \) integrins, we obtained evidence that binding of either one of these integrins to its native ligand induces protein tyrosine phosphorylation in resting human NK cells.

MATERIALS AND METHODS

Antibodies and reagents. The monoclonal antibodies (MoAbs) to \( \alpha_4 \) (P4G9) and \( \alpha_5 \) (P1D6), the synthetic peptides GRGDSP and GRGESP, and the fragments of FN (FN40 and FN120) were pur-
chased from GIBCO, BRL (Gaithersburg, MD). The synthetic peptides EILDVPST (FN CS-1) and EILEVPST (control peptide) were from Peninsula Laboratories (Belmont, CA); unconjugated MoAbs to CD3, CD56, CD16, CD14, and CD19 were from Becton Dickinson (San Jose, CA); anti-paxillin MoAbs (clone 4G10) were from Upstate Biotechnology Inc (Lake Placid, NY); ECL Western blotting detection system was from Amersham (Arlington Heights, IL); and Protein A-Sepharose and Protein G-Sepharose were from Sigma (St Louis, MO).

Purification of human NK cells. Mononuclear cells were isolated by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) gradient centrifugation from leukapheresed products obtained from pistlelet donors (Central Blood Bank of Pittsburgh). To select for peripheral blood lymphocytes (PBLs), monocytes were removed on nylon wool columns as described previously. For all experiments described below, human NK cells were purified from PBLs by negative selection as described earlier. Briefly, PBLs were incubated in the presence of anti-CD3, anti-CD14, and anti-CD15 MoAbs at 1:2 dilution for 30 minutes at 0°C, washed twice, and then incubated with magnetic beads coated with goat-antimouse IgGs (1 cell:30 beads; PerSeptive Diagnostics, Cambridge, MA) for 30 minutes at 0°C. After each of three successive incubations with magnetic beads, a magnet was used to separate beads with attached CD3+-, CD14+-, and CD19+- B cells from NK cells. The cells were then suspended in serum-free RPMI 1640 medium supplemented with 1% heat-inactivated bovine serum albumin (BSA; Sigma) and 25 mmol/L HEPES buffer (GIBCO, Grand Island, NY). By two-color flow cytometry, the enriched NK cell populations contained 94% ± 2% CD3+-, CD56+- (mean ± SEM), 1.0% ± 1% CD3-, 1.0% ± 1% CD19+, and 1.0% ± 0% CD14+- cells.

Flow cytometry. Staining and flow cytometry analyses were performed as previously described using a FACScan (Becton Dickinson) for two-color analysis. Briefly, 2 to 4 × 10^6 cells/tube in phosphate-buffered saline (PBS) containing 0.1% sodium azide were incubated with different combinations of fluorescein- or phycoerythrin-labeled MoAbs for 30 minutes at 0°C. Staining with unlabeled MoAbs was performed by sequential incubation with saturating concentrations of MoAbs to CD3, CD14, and FITC-goat-antimouse IgG (GAMlg) as a second antibody. All MoAbs were preincubated on nonadherent human mononuclear cells to determine optimal dilutions. Results were expressed as the percentage of gated cells positive for each of the MoAbs used. The IgGl and IgG2j isotype controls, second antibody alone, or normal saline controls were included in all experiments.

Adhesion of NK cells to FN-coated surfaces. Adhesion assays were performed in flat-bottom 96-well microtiter plates precoated with 100 μL/well of 25 μg/mL FN (Sigma), or 120- or 40-kd fragments of FN (FN120, FN40; GIBCO) in 0.05 mol/L NaHCO3, pH 9.2 by overnight incubation at 4°C. Subsequently, the coated wells were washed, and unbound sites were blocked by incubation with RPMI supplemented with 1% (wt/vol) heat-inactivated BSA at room temperature for 1 hour. All experiments included control wells coated with 1% BSA only. To evaluate NK cell binding, freshly purified 3Cr-labeled or unlabeled NK cells were added at 1 to 1.5 × 10^5 cells/well in 100 μL RPMI + 1% BSA to the wells. To ensure simultaneous interaction of all cells with the surface-coated FN the cells were sedimented by short centrifugation of the plates (300g, 2 minutes) and then incubated for 0.5 to 3 hours at 37°C. To determine the level of NK cell adhesion to FN, unbound cells were washed off, and the percentage of bound cells was determined by lysing the cells remaining in each well with 1% sodium dodecyl sulfate (SDS) (wt/vol) and counting γ emissions. To determine the effect of antibodies specific for FN receptors on adhesion of NK cells to FN, NK cells were first incubated in the presence of saturating concentrations of MoAb to α1, α2, β1 integrin subunits or anti-CD56 (20 μg/mL) for 30 minutes on ice. To determine the effect of RGD– or CS-1-containing peptides on adherence, predetermined optimal concentration of 0.2 mg/mL of each peptide was added to the substrate-coated wells.

Preparation of cell lysates. To determine whether adhesion to FN induces changes in the basal pattern of phosphorylated proteins, at various time points after centrifugation and incubation of NK cells in wells precoated with FN or FN fragments, unbound cells were washed and the reaction was terminated by the addition of 100 μL/well of cold stop solution (5 mmol/L EDTA, 10 mmol/L sodium fluoride, 10 mmol/L sodium pyrophosphate, and 0.4 mmol/L sodium vanadate in PBS). Control NK cells were incubated in 37°C in the presence of 1% BSA in wells precoated with BSA. Because a very small fraction of NK cells adhered to BSA (2% to 4%), both adherent and nonadherent cells were collected and used as control. Using a plate shaker, FN-adherent NK cells were released into the stop buffer, pelleted, counted, and then solubilized in lysis buffer (1% NP-40, 0.1% deoxycholate, 10 mmol/L EDTA, 100 μg/mL phenylmethylsulfonyl fluoride, 5 μg/mL leupeptin, 10 μg/mL aprotonin, 1 mmol/L sodium vanadate in PBS) for 15 minutes on ice. Insoluble material was removed by centrifugation at 14,000g for 10 minutes and postnuclear supernatant was aliquoted for protein assay (Bio-Rad, Hercules, CA). Aliquots of postnuclear material obtained from a similar number of cells and confirmed by OD280 absorbance to contain similar amounts of protein were mixed with an equal volume of 2× SDS-polyacrylamide gel electrophoresis (SDS-PAGE) buffer, boiled for 5 minutes, and subjected to electrophoresis.

Electrophoresis and immunoblotting. Cell lysates were separated on a 10% or 6% SDS-polyacrylamide gel under reducing conditions according to the method of Laemmli. The separated proteins were then electrophoretically transferred to PVDF membrane (Immobilon-P; Millipore, Bedford, MA). After blocking by 3% solution of nonfat dried milk and 1% BSA for 1 hour at room temperature, the membranes were blotted with antiphosphotyrosine antibodies at a 1:5,000 dilution or with antipaxillin at a 1:1,000 dilution. After washing in TRIS-buffered saline with 0.05% Tween-20 the membranes were incubated with antimouse Ig horseradish peroxidase (1:5,000 dilution; Amersham, Arlington Heights, IL) for 1 hour. Protein bands were detected by enhanced chemiluminescence (ECL: Amersham).

Immunoprecipitation analysis. Lysates were centrifuged at 14,000g for 10 minutes and precleared with 30 μL of a 50% suspension of protein G-Sepharose CL-4B for 2 hours at 4°C. Precleared cell lysates were incubated with antiphosphotyrosine or antipaxillin MoAbs for 4 to 16 hours at 4°C and immune complexes were bound to protein G-Sepharose during a 2-hour incubation at 4°C. The protein G-bound immune complexes were precipitated, washed with lysis buffer, resuspended in SDS-PAGE sample buffer, boiled for 5 minutes, and analyzed by SDS-PAGE.

Statistical analyses. Significance of the observed differences was calculated using Mann-Whitney U test for nonpaired samples or Wilcoxon’s rank sum test for paired data. These analyses were performed with the aid of the StatView II program (Abacus Concepts Inc, Berkeley, CA).

RESULTS

Induction of protein tyrosine phosphorylation by adhesion of NK cells to immobilized FN. To study the effect of interactions of the integrins expressed on resting human NK cells with FN, we examined protein phosphorylation on tyrosine,
Tides. NK cells were incubated in FN-coated wells in the presence or absence of MoAb. The position 37°C in the presence of FN in the presence of RGD-, RGE- or CS-l-containing pep- proteins to Immobilon-P and Western blotting with antiphytYro- on the left. The results shown are representative of at least six experi- ments.

The integrin α2β1 interacts with RGD sequences present in FN molecule, whereas ααβ1 binds to CS-1 FN fragment via EILDVPST sequence. The results presented in Fig 2 show the inhibition of NK cell adhesion to FN at a level of 70% in the presence of anti-β1 MoAb, suggesting that the β1 integrins are the major FN receptors used by NK cells for attachment to FN. The presence of either GRGDSP or EILDVPST peptides inhibited significantly FN-mediated tyrosine phosphorylation. In contrast, the presence of the GRGESp control peptide had no effect on FN-mediated tyrosine phosphorylation.

Inhibition of NK cell attachment to FN and FN-induced protein tyrosine phosphorylation by RGD- or CS-1-containing peptides. To explore the role of individual integrins in FN-induced elevated level of phosphorytosine proteins in NK cells, we first examined their relative contribution to NK cell attachment to FN. NK cells were allowed to adhere to FN in the presence of GRGDSP or EILDVPST peptides. The integrin α2β1 interacts with RGD sequences present in FN molecule, whereas ααβ1 binds to CS-1 FN fragment via EILDVPST sequence. The results presented in Fig 2 show the inhibition of NK cell adhesion to FN at a level of 70% in the presence of anti-β1 MoAb, suggesting that the β1 integrins are the major FN receptors used by NK cells for attachment to FN. The presence of either GRGDSP or EILDVPST peptides inhibited significantly (P < .05), though partially, the attachment of NK cells to FN as measured by adhesion of 51Cr-labeled NK cells. The relative contribution of each of the receptors, α2β1 or ααβ1, appears to be equivalent, because similar inhibition was observed by blocking interactions with FN of either α4 or α5 integrins by MoAbs or peptides. However, binding of NK cells to immobilized 120-kD chymotryptic fragment of FN, which contains the central cell-binding domain (RGD) but lacks the heparin-binding domain, was mediated by ααβ1, and not affected by GRGESp control peptide, anti-α4, or CS-1 pep-
tide. Conversely, adhesion of NK cells to an immobilized 40-kD fragment that contains the alternative spliced region (CS-1) was inhibited by EILDVPST peptide or anti-α2 integrin subunit, but not by GRGDSP or EILEVPST peptides.

To investigate the kinetics of protein tyrosine phosphorylation induced by ligation of each of these β1 integrins, NK cells adherent to FN for 0.5 to 3 hours were lysed, and equal amounts of protein were electrophoresed on SDS-polyacrylamide gel, transferred to Immobilon-P membrane, and probed with antiphosphotyrosine MoAb. A marked increase in phosphotyrosine content was observed with increasing time of NK cell adhesion to FN, compared with control NK cells (Fig 3A). Because NK cells interact with FN via α2β1 and/or α5β1 integrins, the observed increases in phosphotyrosyl proteins could have been induced by triggering through either one or both of these FN receptors. Western blotting of NK cells plated on immobilized FN in the presence of the inhibitory peptides, CS-1 (Fig 3A), showed reduced intensity of phosphotyrosine bands mainly at an early time point (30 minutes) of adhesion compared with NK cells plated in the absence of any inhibitory peptide. However, when the profile of phosphotyrosine proteins was examined after 2 hours of adhesion, less inhibition than that after 30 minutes of adhesion was observed in the presence of CS-1. In NK cells adherent to FN for 30 minutes in the presence of RGD-containing peptides, a reduced level of phosphotyrosine proteins was observed compared to cells treated with control RGE peptides (Fig 3B). However, already at 1 hour of adhesion, no inhibition was observed compared with NK cells adhering to FN in the presence of control RGE-containing peptides. Because either CS-1 or RGD-containing peptides inhibited phosphorylation primarily during early adhesion, it appears that binding of FN to both α2β1 and α5β1 FN receptors on NK cells contributed to the increased tyrosine phosphorylation. The increased phosphorylation observed after 2 hours of adhesion in the presence of a single inhibitory peptide, either CS-1 or RGD, might be related to triggering via the reciprocal unblocked FN receptor.

Induction of protein tyrosine phosphorylation in NK cells by immobilized FN fragments. To further investigate the capability of α2β1 or α5β1 integrins on NK cells to trigger protein tyrosine phosphorylation, we have examined the involvement of different segments of FN in induction of kinase activity. The 120-kD chymotryptic fragment of FN (RGD-binding domain) and a 40-kD fragment (EILDVPST-binding domain) were immobilized on plastic surfaces and used as adhesive substrates for NK cells. In NK cells adherent to FN120 or FN40, and subsequently analyzed by Western blotting with antiphosphotyrosine, both FN fragments induced a time-dependent increase in tyrosine phosphorylated proteins (Fig 4). However, using immobilized fragments of FN as adhesive substrate for NK cells, a lower number of phosphoryltyrosyl intracellular proteins was detected. After binding to either FN40 or FN120, the induced tyrosine phosphorylation was mainly associated with 120- and 70-kD proteins. It is possible that engagement of a single integrin on the surface of NK cells would transduce quantitatively and/or qualitatively different signal than that transduced by occupancy of both FN receptors. This concept is supported by studies showing that full adhesive and migratory function of cells require the cooperativity or synergy between FN adhesion sites.77

Induction of tyrosine phosphorylation of paxillin in NK cells adherent to FN. Since paxillin was reported to be a substrate for tyrosine kinases in fibroblasts during focal contact formation,28,30 we tested whether the 70-kD protein whose state of tyrosine phosphorylation increased during NK cell adhesion to FN was paxillin. Immunoprecipitation of paxillin, followed by immunoblotting with antiphosphotyrosi-
sine, showed enhanced staining of a 70-kD protein, the predicted molecular mass of paxillin, relative to control NK cells incubated in BSA-pretreated wells (Fig 5A). To confirm that paxillin undergoes enhanced tyrosine phosphorylation in NK cells adherent to FN, lysates of adherent cells were subjected to immunoprecipitation of tyrosine phosphoproteins, followed by immunoblotting with antipaxillin (Fig 5B). Control immunoprecipitation by antipaxillin followed by blotting by antipaxillin (Fig 6) showed that a similar quantity of paxillin was detected in NK cells treated with FN or BSA. These findings confirmed the identification of paxillin as a major tyrosine phosphorylated protein in FN-induced NK cells. However, our results showed that at least one additional protein was immunoprecipitated from BSA- or FN-treated cells by antiphosphotyrosine and visualized by antipaxillin (Fig 6). In preliminary studies we have found kinase activity associated with paxillin immunoprecipitate. These findings may imply that stable association exists between this focal adhesion protein and tyrosine kinases. Experiments to resolve this issue are now in progress.

DISCUSSION

Taken together, our results indicate that activation of protein tyrosine kinases, which results in tyrosine phosphorylation of several intracellular proteins, is triggered by β1 integrin receptors expressed on resting NK cells. These signals may be of significance in adhesion-induced changes in NK cell behavior within a tissue microenvironment. Unlike growth factor receptors that initiate signaling through the intrinsic protein tyrosine kinase activity of their cytoplasmic

**Fig 4.** Induction of tyrosine phosphorylation in NK cells after adhesion to plastic-immobilized FN40 (A) and FN120 (B). Lysates prepared from purified NK cells plated for 0.5 to 3 hours on surfaces coated with FN or FN fragments were electrophoresed on polyacrylamide gels, transferred to Immobilon-P membranes, and immunoblotted with antiphosphotyrosine. Control cells were incubated in BSA-treated wells for 1 hour at 37°C. Results of one representative experiment of three performed are presented.

**Fig 5.** Tyrosine phosphorylation of paxillin in FN-adherent NK cells. NK cells adherent to FN for 30 minutes in 37°C or control NK cells incubated in BSA-pretreated wells were lysed and precleared with protein G-Sepharose for 2 hours. Lysates of parallel samples were immunoprecipitated with either antipaxillin or antiphosphotyrosine MoAbs and the immunoprecipitates were analyzed by Western blotting with antiphosphotyrosine or antipaxillin MoAbs, respectively.
tails, most integrins have a short cytoplasmic domain and do not have any obvious homologies with G protein-coupled receptors. Thus, the process of tyrosine phosphorylation is probably initiated by integrins indirectly, perhaps by modifying the activity or subcellular localization of nonreceptor tyrosine kinases, tyrosine phosphatases, or their substrate.

Our data show that of and on human NK cells by FN rapidly stimulated tyrosine phosphorylation, mainly on proteins with a high molecular mass. It is likely that these proteins are associated with sites of adhesion to the ECM known as "focal adhesions." In cultured cells, integrins have been shown to cluster at focal adhesions that are thought to be important not only as structural links between the ECM and the cytoskeleton, but also as sites of signal transduction from the ECM. Several potential regulatory proteins have been identified in focal adhesions, including protein kinase C, tyrosine kinases, and a new tyrosine kinase, pp125FAK (focal adhesion kinase), which are implicated in regulating the integrity of these structures. Paxillin is a 70-kD protein originally identified in chicken embryonic fibroblasts. It is localized to focal adhesions and binds to actin-capping protein, vinculin. Like pp125FAK kinase, paxillin is a major phosphoryl protein in chicken embryonic fibroblasts transformed by Rous sarcoma virus and is also tyrosine phosphorylated during chick embryonic development and in rat embryos. Recently it was reported that tyrosine phosphorylation of paxillin as well as pp125FAK kinase occurs as a consequence of integrin activation induced by plating fibroblasts on fibronectin-coated surfaces. The role of paxillin in lymphocytes has not yet been studied. Our findings identify paxillin as a substrate of integrin-stimulated tyrosine phosphorylation in NK cells. The phosphorylation of paxillin may serve to link integrin-mediated signaling events with dynamic changes in the cytoskeleton. Our preliminary studies indicate that kinase activity is associated with paxillin immunoprecipitates. These findings may imply that stable association exists between this focal adhesion protein and tyrosine kinases. Increasing numbers of studies have recently demonstrated physical association of various kinases with T-cell antigen receptor, B-cell antigen receptor, and Fc receptors. Furthermore, stable association of p59 with the focal adhesion protein pp125FAK has been shown in normal cells. Also, tyrosine phosphorylated-paxillin has been shown to generate high-affinity interactions with SH2 or SH3 domains of virus-encoded fusion protein, v-Crk, which result in elevated tyrosine phosphorylation in transformed cells. Thus, the state of phosphorylation of tyrosine residues on paxillin may have a role in its interaction with other cytoskeleton proteins or downstream kinases.

It has been reported that the major p120 protein phosphorylated on tyrosine after plating NIH 3T3 cells on FN or anti-integrin β1 antibody is identical to pp125FAK, at least on the basis of immunologic crossreactivity with anti-pp125FAK antibody. In lymphoid cells, clustering of VLA-4 on T cells with anti-β1 antibodies or on B cells with either antibodies or the two ligands, FN40 or VCAM-1, triggered tyrosine phosphorylation of 105-kD protein. However, this protein has not yet been identified. Experiments designed to determine the possible relationship between the high-molecular-weight proteins and pp125FAK are now in progress. Like paxillin, pp125FAK may be a component of a signal transduction pathway responsible for the transmission of signals from the cell-surface integrins into the cell. Alternatively, it may be responsible for phosphorylating the components of focal adhesions. There seem to be parallels between signaling events triggered in lymphoid cells via nonintegrin receptors with relatively short cytoplasmic tails such as CD4 and CD8, which interact with src family kinases, and events related to integrin-mediated phosphorylation. Because oncogenic and growth factor-associated protein tyrosine kinases were shown to play a crucial role in regulation of cell functions, it is likely that they would also be involved in regulatory effects of integrins in NK cells.

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Induction of protein tyrosine phosphorylation in human natural killer cells by triggering via alpha 4 beta 1 or alpha 5 beta 1 integrins

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