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IL-2 Induces STAT4 Activation in Primary NK Cells and NK Cell Lines, But Not in T Cells

Kathy S. Wang, Jerome Ritz, and David A. Frank

IL-2 exerts potent but distinct functional effects on two critical cell populations of the immune system, T cells and NK cells. Whereas IL-2 leads to proliferation in both cell types, it enhances cytotoxicity primarily in NK cells. In both T cells and NK cells, IL-2 induces the activation of STAT1, STAT3, and STAT5. Given this similarity in intracellular signaling, the mechanism underlying the distinct response to IL-2 in T cells and NK cells is not clear. In this study, we show that in primary NK cells and NK cell lines, in addition to the activation of STAT1 and STAT5, IL-2 induces tyrosine phosphorylation of STAT4, a STAT previously reported to be activated only in response to IL-12 and IFN-α. This activation of STAT4 in response to IL-2 is not due to the autocrine production of IL-12 or IFN-α. STAT4 activated in response to IL-2 is able to bind to a STAT-binding DNA sequence, suggesting that in NK cells IL-2 is capable of activating target genes through phosphorylation of STAT4. IL-2 induces the activation of Jak2 uniquely in NK cells, which may underlie the ability of IL-2 to activate STAT4 only in these cells. Although the activation of STAT4 in response to IL-2 occurs in primary resting and activated NK cells, it does not occur in primary resting T cells or mitogen-activated T cells. The unique activation of the STAT4-signaling pathway in NK cells may underlie the distinct functional effect of IL-2 on this cell population. The Journal of Immunology, 1999, 162: 299–304.

Natural killer cells are large granular lymphocytes that are distinct from T cells and other lymphocytes in the immune system. NK cells play an important role in antiviral immunity and in defense against tumor cells. They exhibit spontaneous cytotoxic activity toward cells that do not express class I MHC molecules, a common consequence of viral infection or tumor transformation, and thus they are important cellular components of innate immunity (1). The development and functional activity of NK cells are regulated by many cytokines, including IL-2, a cytokine initially identified as T cell growth factor (2). IL-2 stimulates the proliferation of both T cells and NK cells (3–7). However, in NK cells, IL-2 has the additional effect of augmenting cytotoxic function (8–10), similar to the effect of IL-12.

IL-2 mediates its effects through interaction with a multichain cell surface receptor and activation of the Janus kinase (Jak)/STAT pathway (11). Upon binding to its receptor, IL-2 induces tyrosine phosphorylation of two members of the Jak family, Jak1 and Jak3 (12, 13). In T cells, NK cell lines, and activated NK cells, the phosphorylation of Jak1 and Jak3 leads to the recruitment and activation of STAT1, STAT3, and STAT5, which then translocate to the nucleus and activate target genes (14–16). Since these signaling events are identical in NK cells and T cells, it is likely that these pathways mediate effects of IL-2 that are common to both cell populations, such as proliferation. That IL-2 enhances cytotoxicity in NK cells, but not in T cells suggests that an alternative IL-2 signaling pathway may exist that is unique to NK cells.

To explore this hypothesis, we investigated alternative pathways of IL-2 signaling in primary NK cells and in two NK cell lines. In addition to activating STAT1 and STAT5, we found that IL-2 activates STAT4, a STAT that was previously reported to be activated only in response to IL-12 and IFN-α. Although IL-2 can activate STAT1 and STAT5 in T cells, it does not activate STAT4 in these cells. IL-2 also leads to the activation of Jak2 only in NK cells. Thus, the selective activation of Jak2 and STAT4 in response to IL-2 in NK cells may explain the enhancement of cytolytic activity of these cells by IL-2.

Materials and Methods

Cytokines and Abs

Human rIL-2 (specific activity, 6 × 10^6 U/ml) was provided by Amgen (Thousand Oaks, CA). Human rIL-12 (specific activity, 1.7 × 10^7 U/mg) was kindly provided by Genetics Institute (Cambridge, MA). Human rIFN-α was obtained from Hoffman-La Roche (Nutley, NJ). Purified IL-4 (specific activity, 1 × 10^7 U/mg) was purchased from Genzyme (Cambridge, MA). Purified unconjugated and phycoerythrin (PE)-conjugated murine mAbs, including B1 (CD20, IgG2a), My4 (CD14, IgG2b), NKH1 (CD56, IgG1), T1 (CD5, IgG2a), T3 (CD3, IgG1), and MdIgG1, were obtained from Coulter Immunology (Miami, FL). Anti-phosphotyrosine Ab PY99, and STAT4, Tyk2, Jak1, Jak2, and Jak3 Abs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The generation of Abs to tyrosine-phosphorylated STAT1 (14) (which also cross-reacts with tyrosine-phosphorylated STAT5 (17, 18)), and the IL-12 receptor β1 subunit, 12Rß44 (19), were described previously. Anti-IFN-α neutralizing Ab was generously provided by Endogen (Woburn, MA).

Reagents

PMA and FCS were obtained from Sigma (St. Louis, MO). Ionomycin was purchased from Calbiochem (La Jolla, CA). [γ-32P]ATP was purchased from New England Nuclear Life Science Products (Boston, MA). Lymphocult was obtained from Biotest Diagnostics (Denville, NJ).

Purification and culture of NK cells and T cells

PBMCs were isolated from heparinized blood obtained from normal donors by ficoll-hypaque (Pharmacia LKB, Piscataway, NJ) gradient centrifugation. Primary NK cells (>96% purity) were obtained by first depleting monocytes, B cells, and T cells from PBMCs with B1 (CD20,
IgG2a), My4 (CD14, IgG2b), T1 (CD5, IgG2a), and T3 (CD3, IgG1) Abs and immunomagnetic anti-IgG beads (Advanced Magnetics, Cambridge, MA). The remaining cells were incubated with PE-conjugated CD56 and sorted on an EPICS Elite ESP flow cytometer (Coulter). Activated NK cells were obtained by culturing primary NK cells for 4 days in RPMI 1640 medium containing 15% FCS and 10% Lymphocult, with 750 ng/ml ionomycin included for the first 16 h of culture.

Primary T cells (>98% purity) were obtained by incubating PBMCs with PE-conjugated CD5 and sorting by flow cytometry. PHA-activated T cells were prepared by culturing purified primary T cells for 4 days in RPMI 1640 medium containing 15% FCS and 2.5 μg/ml PHA (Murex, Dartford, U.K.). PHA-activated T cells used for immunoprecipitation experiments (>94% purity) were purified by depleting monocytes, B cells, and NK cells from PBMCs with My4 (CD14, IgG2b), B1 (CD20, IgG2a), and NKLH (CD56, IgG1) Abs and immunomagnetic anti-IgG beads.

NK3.3 cells (20) were cultured in RPMI 1640 medium containing 15% FCS, 10% Lymphocult, and 50 U/ml IL-2. NKL cells (21) were cultured in RPMI 1640 medium containing 15% FCS and 50 U/ml IL-2.

Whole cell extracts and Western blotting

Following stimulation, cells were washed once with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM NaH2PO4, 1.8 mM KH2PO4 (pH 7.4)) and extracted with EBC 250 buffer containing 0.5% Nonidet P-40, 250 mM NaCl, 10 mM Tris (pH 8), 10 mM sodium orthovanadate, 100 μM PMSF, 1 μg/ml pepstatin, and 1 μg/ml aprotinin. Insoluble material was removed by centrifugation at 12,000 × g for 5 min. Protein was separated on a 9% gel by SDS-PAGE and subsequently electrotransferred to nitrocellulose. The membrane was blocked with either 5% BSA (for phosphotyrosine blots) or 5% dry milk in TBST (100 mM Tris-HCl (pH 8), 150 mM NaCl, and 0.05% Tween 20), and probed with primary Ab diluted 1/10,000. After incubation with horseradish peroxidase-conjugated secondary Ab, specific protein was detected by chemiluminescence (ECL; New England Nuclear Life Science Products).

Immunoprecipitation

Whole cell extracts were precleared with rabbit anti-mouse Ig-coupled protein A beads, followed by a 1-h incubation at 4°C with anti-STAT4, anti-Jak2, or anti-Tyk2 Ab and 1 h with rabbit anti-mouse-coupled protein A beads. After extensive washing, beads were boiled in sample buffer containing 2-ME; precipitated protein was resolved by SDS-PAGE.

Nuclear extracts and electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared as described previously (22). Nuclear extract (2 μl) was mixed with 1 ng of 32P-labeled oligonucleotide in 10 μl of binding buffer (25 mM HEPES (pH 7.9), 100 μM EDTA, 200 μM MgCl2, 500 μM DTT, 1 mg/ml BSA, 0.4 mg/ml double-stranded poly (dI:dC), 30% glycerol, and 0.1 mg/ml salmon sperm DNA) and incubated at room temperature for 15 min. The STAT-binding oligonucleotide used, 5′-GAGCG CTGTATTCCTCCGAAATGATGACGGT-3′ and its complement, is derived from the IFN-γ-responsive factor 1 gene promoter (23). After incubation at room temperature, 1 μl of anti-STAT4 Ab was added where indicated for supershift analysis, and samples were incubated on ice for an additional 15 min. The protein-DNA complexes were separated on a 4% acrylamide gel in 0.5X TBE (45 mM Tris-borate, 1 mM EDTA) and visualized by autoradiography.

Results

IL-2 activates STAT4 in NK cell lines

To explore alternative pathways for IL-2 signaling in NK cells, we examined the activation of different STATs in response to IL-2 in two human NK cell lines, NKL and NK3.3. Both of these cell lines are IL-2 responsive and require IL-2 to maintain growth in vitro. While IL-2 treatment induced STAT1 and STAT3 activation, as reported previously (14, 16) (data not shown), it also induced forms of STAT4 with slow migration in both NK cell lines (Fig. 1, A and B, lane 2). IFN-α, which was shown to activate tyrosine phosphorylation of STAT4 in NK3.3 cells (24), also produced slow migration STAT4 bands in both NK cell lines (Fig. 1A, lane 4, and B, lane 5). IL-12, which is known to induce tyrosine phosphorylation of STAT4 in NK3.3 cells, also led to the formation of slow migration forms of STAT4 in these cells (Fig. 1B, lane 4). NKL cells lack expression of the β2 subunit of the IL-12 receptor, as assessed by reverse-transcriptase PCR, and do not respond to IL-12 (data not shown). No slow migration forms of STAT4 were observed in untreated cells (Fig. 1, A and B, lane 1) or in IL-4-treated cells (Fig. 1, A and B, lane 3).

Previous studies have demonstrated that phosphorylation of STAT4 is associated with slowed electrophoretic migration of this protein (24, 25). Since tyrosine phosphorylation is critical to the activation of STATs, we examined directly whether IL-2 induces tyrosine phosphorylation of STAT4. NK3.3 cells were left untreated or treated with specific cytokines, and whole cell extracts were prepared. STAT4 was immunoprecipitated, and tyrosine phosphorylation was assessed by Western blotting with anti-phosphotyrosine Ab 8Y99. Whereas STAT4 was unphosphorylated in resting NK3.3 cells (Fig. 2A, lane 1), IL-2, IL-12, and IFN-α all induced tyrosine phosphorylation of STAT4. The degree of STAT4 tyrosine phosphorylation induced by IL-2 was greater than that of IL-12 and was comparable with that induced by IFN-α. Reprobing with an Ab to STAT4 revealed that tyrosine-phosphorylated STAT4 (Fig. 2A) induced by IL-2, IL-12, or IFN-α phosphorylates STAT4 (Fig. 2A) induced by IL-2, IL-12, or IFN-α.
activation of STAT4 in response to exogenous IFN-α in the absence or presence of neutralizing Abs against IFN-α (A), or the β1 subunit of the IL-12R (B). Western blotting was performed with Ab to STAT4.

migrated as the band with slowest mobility (Fig. 2B). The intermediate bands most likely represent STAT4 phosphorylated on serine residues ((24) and data not shown).

**FIGURE 3.** IL-2 activation of STAT4 does not involve IL-12 or IFN-α. NK3.3 cells were untreated or treated with cytokines, as indicated, in the presence or absence of neutralizing Abs against IFN-α (lane 2, 4, and 6). Western immunoblotting was performed with Ab to STAT4.

IL-2-induced STAT4 activation does not involve autocrine production of IL-12 or IFN-α

STAT4 has been reported to be activated only in response to IL-12 in NK cells and T cells (24–26), and IFN-α in NK3.3 cells (24). Although IL-2 treatment of NK cells is not known to lead to production of IL-12 or IFN-α, we considered the possibility that autocrine production of one of these factors might lead to STAT4 phosphorylation. NK3.3 cells were treated with cytokines in the presence or absence of Abs that block IL-12 or IFN-α signaling. The presence of an IFN-α-neutralizing Ab, which abolished the activation of STAT4 in response to exogenous IFN-α (1000 U/ml) (Fig. 3A, lane 5), did not affect the activation of STAT4 in response to IL-2 (Fig. 3A, lane 3). Similarly, STAT4 activation in response to IL-2 was not affected by the presence of an Ab against the β1 subunit of the IL-12R (12Rβ1), which could block the ability of IL-12 to activate STAT4 phosphorylation (Fig. 3B). These results indicate that the STAT4 activation in response to IL-2 is not due to exogenous stimulation with IL-12 or IFN-α.

***IL-2 activates STAT4 in primary resting and activated NK cells, but not in primary resting or activated T cells***

Although the NK cell lines NKL and NK3.3 exhibit many properties of NK cells, through adaptation to in vitro growth, they may display differences from primary NK cells. To test whether IL-2 activates STAT4 in primary human NK cells, NK cells were purified from PBMCs of healthy donors by FACS. NK cells were activated by ionomycin for 16 h, and IL-2 plus Lymphocult for 3 days, then cells were rested overnight and treated with specific cytokines. Whole cell extracts were prepared and Western blotting was performed. As indicated by the appearance of the slow migrating STAT4 band, IL-2 could activate STAT4 in these activated NK cells (Fig. 4A, lane 2). To investigate whether IL-2 could also activate STAT4 in activated T cells, T cells were isolated from PBMCs of healthy donors and activated for 4 days with PHA, then washed and rested overnight. In these activated T cells, IL-12 and IFN-α induced slow migration forms of STAT4 (Fig. 4B, lanes 4 and 5), consistent with tyrosine phosphorylation of this protein. By contrast, IL-2 did not induce any change in the mobility of STAT4 in these cells (Fig. 4B, lane 2). That these activated T cells could respond to IL-2 was indicated by the tyrosine phosphorylation of STAT1 and STAT5 induced by this cytokine (Fig. 4C, lane 2). When extracts from cytokine-treated activated T cells were analyzed by immunoprecipitation with Abs to STAT4 and probed with PY99, it was confirmed that IL-12 or IFN-α, but not IL-2, induced tyrosine phosphorylation of STAT4 (Fig. 5). These results suggest that IL-2 activates STAT4 in activated NK cells, but not in activated T cells. To test whether similar signaling occurs in resting primary NK cells and T cells, IL-2-induced phosphorylation of STAT4 was compared in NK cells and T cells that were purified from the same healthy donor. In cells from four individuals, IL-2 induced STAT4 activation in primary NK cells (Fig. 6A, upper panel, lane 2), but not in the primary T cells from the same donor (Fig. 6B, upper panel, lane 2). Reprobing the membrane with anti-phosphotyrosine-STAT1 Ab showed that IL-2 activates STAT1 in both primary NK cells and primary T cells (Fig. 6A, and B, lower panels, lane 2), indicating that these cells can respond to this cytokine. Activation of STAT5 in response to IL-2 also occurred in primary T cells (Fig. 6B, lower panel, lane 2). These results suggest that the lack of STAT4 activation in response to IL-2 in primary resting T cells or in activated T cells is not due to an absence of IL-2 responsiveness in these cells. From these data, we conclude that, as in NK cell lines, IL-2 activates STAT4 in primary resting and activated NK cells. Moreover, the activation of STAT4 by

**FIGURE 4.** IL-2 activates STAT4 in activated NK cells, but not in activated T cells. Activated NK cells (A) and activated T cells (B and C) were untreated or treated with the indicated cytokine for 20 min. Western blots were performed with Ab to STAT4 (A and B) or tyrosine-phosphorylated STAT1 (C). C represents a reprobing of the membrane in B.

**FIGURE 5.** IL-2 does not induce tyrosine phosphorylation of STAT4 in activated T cells. Activated T cells were untreated or treated with the indicated cytokine for 20 min. Immunoprecipitation of STAT4 was performed on whole cell extracts, followed by Western blotting with Ab to phosphotyrosine (A). After stripping, the membrane was reprobed with Ab to STAT4 (B).
IL-2 is restricted to NK cells, and is not found in either primary or activated T cells.

IL-2-activated STAT4 is capable of binding DNA

The number of primary NK cells required for immunoprecipitation exceeds that which can be obtained by cell sorting. Thus, to confirm the activation of STAT4 in primary NK cells, DNA-binding assays were performed. Once activated by tyrosine phosphorylation, STATs dimerize, translocate to the nucleus, bind to specific DNA sequences in promoter regions, and activate the transcription of their target genes. Therefore, if STAT4 phosphorylated in response to IL-2 is functionally active, it must translocate to the nucleus and bind to cognate DNA sequences. To investigate whether STAT4 activated by IL-2 in NK cells is in the nucleus and capable of binding DNA, nuclear extracts were prepared from untreated or cytokine-treated activated NK cells, and EMSA was performed. The oligonucleotide used in the EMSA is derived from the IFN-γ-responsive factor-1 gene promoter, and can bind multiple STATs, including STAT1, STAT3, STAT4, and STAT5 (14, 23, 27). As indicated in Fig. 7A, IL-2 induced three protein-DNA complexes, A, B, and C. By contrast, IL-12 induced only complex B, and IFN-α induced complexes A and C. It has been shown previously that in NK cells, IL-12 activates STAT4, and IFN-α activates STAT4 and STAT1. Given our finding that IL-2 activates STAT1, STAT5, and STAT4, the common complex induced by all three cytokines, complex A, would be most likely to include STAT4. Indeed when Ab to STAT4 was introduced into the binding mixture, complex A disappeared. Instead, a supershifted complex, complex S, appeared in all three cytokine conditions. Complex C, which was induced by IL-2 and IFN-α, has been found to contain STAT1 (data not shown). Complex B, which was formed only in response to IL-2, most likely contains STAT5. These data indicate that STAT4 activated in response to IL-2, like STAT4 activated by other cytokines, is capable of translocating to the nucleus and binding to target DNA sequences. To verify further that IL-2 does not induce STAT4 activation in T cells, EMSA was performed with nuclear extracts from activated T cells that were untreated or treated with cytokines. While IL-12 and IFN-α induced complexes in activated T cells similar to those in activated NK cells, IL-2 did not induce formation of complex A, which contains activated STAT4 (Fig. 7B). This indicates that in contrast...
to activated NK cells, IL-2 does not induce DNA-binding activity of STAT4 in activated T cells.

**IL-2 activates Jak2 tyrosine kinase in NK cell lines, but not in activated T cells**

Upon binding to their receptors, cytokines induce phosphorylation and activation of members of the Jak family, which leads to the recruitment and phosphorylation of STATs. It has been shown that Jak2 and/or Tyk2 are involved in the IL-12 and IFN-α signaling events that activate STAT4, in contrast to the kinases reported to be activated by IL-2, Jak1, and Jak3. To investigate whether Jak2 or Tyk2 is involved in the activation of STAT4 in response to IL-2 in NK cells, Jak2 and Tyk2 were immunoprecipitated from NKL, NK3.3, and activated T cells that had been untreated or treated with cytokines. The phosphorylation of Jak2 and Tyk2 was assessed by performing a Western blot with anti-phosphotyrosine Ab to phosphotyrosine (A and B, upper panel). In contrast, IFN-α led to the activation of Tyk2 in NKL and NK3.3 cells, and both IL-12 and IFN-α caused activation of Tyk2 in activated T cells. However, IL-2 did induce phosphorylation of Jak2 in both NK cell lines, but not in activated T cells (Fig. 8B, upper panel). These data suggest that Jak2, but not Tyk2, may be involved in the activation of STAT4 in response to IL-2 in NK cells.

**Discussion**

One of the key unanswered questions in the regulation of hemopoietic function is how cytokines can exert distinct effects on diverse cell populations. For example, while IL-2 can induce proliferation of both T cells and NK cells, it has the distinct action of augmenting the cytotoxic function of NK cells. The potentiation of cytotoxicity by IL-2 is similar to the effect that IL-12 exerts on NK cells, although IL-12 is much less potent at inducing proliferation of NK cells. This suggests that IL-2 can induce a variety of intracellular signaling events in T cells and NK cells, some of which overlap (such as those controlling proliferation), and some of which are distinct (such as those controlling cytotoxicity). In NK cells, IL-2 and IL-12 exert some overlapping effects (such as the augmentation of cytotoxicity), and some that are distinct (such as the stimulation of proliferation). Thus, it would be expected that IL-2 and IL-12 would share some, but not all, of the intracellular pathways activated by each cytokine. To dissect the mechanisms that underlie these dichotomies, we analyzed the activation of STAT transcription factors in response to IL-2 in purified populations of normal resting or activated T cells and NK cells, and in NK cell lines. We found that whereas IL-2 could activate STAT1 and STAT5 in both cell types, IL-2 had the unique ability to induce STAT4 activation only in NK cells.

This finding is significant in that it suggests that one mechanism by which a single cytokine can induce unique effects in different cell types is to activate distinct signaling pathways in each. Cell-restricted signaling activation would allow a relatively small group of cytokines to exert distinct effects on various cell types. Cells of clearly disparate lineages may have other mechanisms for modulating the subset of genes activated by a given cytokine. For example, gene silencing through DNA methylation or other mechanisms may restrict the potential target genes activated by a cytokine. In more closely derived cell types, such as T cells and NK cells, such a mechanism may not be practical, as similar genes may ultimately need to be activated at some time in each. Thus, restricting the transcription factors that can be activated in a specific cell type would allow an easily modified mechanism to alter gene activation in response to a given cytokine.

The STAT family of transcription factors appears to play an important role in mediating gene activation by most hemopoietic, and many nonhemopoietic cytokines, growth factors, and neurotrophic factors (28). Some members, such as STAT1, STAT3, and STAT5, are activated in response to a very small number of cytokines. These proteins may be involved in mediating more general types of signals, such as those for cell growth and survival. Others, including STAT4, are more restricted in their expression (29), and are activated in response to a very small number of factors. This may reflect a more specialized role for these STATs. The loss of STAT4 appears to have a major impact on the differentiation of Th subsets (30, 31), indicating that one of these roles most likely involves T cell differentiation. The findings presented in this study would suggest that STAT4 is also important in mediating the effects of IL-2 on NK cells, perhaps in the activation of cytotoxicity. This cell type restriction of STAT activation is an attractive model for increasing the diversity of cellular responses induced by a given cytokine.

To determine the mechanism by which IL-2 induces STAT4 activation only in NK cells, we examined activation of Jak family members in response to IL-2 in these cells. While IL-2 is known to induce the activation of Jak1 and Jak3, we considered whether IL-2 might activate another Jak family member only in NK cells. Although specificity in STAT signaling is not thought to be restricted by the kinase that is activated, it is conceivable that in this system a kinase might influence the choice of substrate. In fact, we found that IL-2 induced the activation of Jak2 in NK cells, but not in T cells. This unique signaling event in NK cells might underlie
the ability of IL-2 to activate STAT4 only in this population, although the mechanism by which this occurs is unclear. One hypothesis is that an additional receptor chain is found associated with the IL-2R in NK cells, but not in T cells, and this mediates activation of Jak2 and STAT4. For example, recruitment of one or more chains of the IL-12R by the IL-2R in NK cells might allow Jak2 to be activated and STAT4 to become phosphorylated uniquely in NK cells. Such a common receptor chain would be similar to the β2 chain shared by the receptors for granulocyte-macrophage colony-stimulating factor (CSF), IL-3, and IL-5. These cytokines can compete for this signal-transducing chain on the cell surface, and the β2 chain confers signaling competency to the cognate ligand of the receptor α-chain with which it associates. Such a common receptor chain would allow IL-2 to replicate at least some of the signaling events induced by IL-12 in NK cells.

There are other mechanisms for recruitment of Jak2 and/or STAT4 to IL-2R complexes. For example, a novel docking protein whose expression is restricted to NK cells might allow the recruitment of these molecules to the IL-2R in NK cells, but not in T cells. STAT proteins are believed to interact with tyrosine-phosphorylated receptor-kinase complexes via their SH2 domain. Thus, the presence of a protein in NK cells that associates with the IL-2R and can become tyrosine phosphorylated at a site that is recognized by the STAT4 SH2 domain would allow STAT4 to become phosphorylated in response to IL-2 uniquely in NK cells.

While the stimulation of cytotoxicity is a known effect of IL-2 on NK cells, it is possible that IL-2 also affects NK cells in other ways. For example, IL-2 may alter the expression of cell surface molecules or the survival of NK cells. These effects of IL-2 may all be mediated by STAT4 activation, although it is possible that other signaling pathways might also be regulated by IL-2 in NK cells. While the principal substrates for the Jak family of kinases are believed to be STAT transcription factors, it is possible that Jak2 may phosphorylate other proteins in NK cells. Thus, in addition to STAT4, the activation of Jak2 might lead to signaling events through other pathways that might further modulate NK cell function.

In conclusion, the restriction of STAT4 activation to NK cells sheds light on the differential response of NK cells and T cells to IL-2. Importantly, this finding provides a potential mechanism by which a single cytokine can have overlapping but distinct effects on two critical effector cells. Furthermore, it suggests that the recruitment and phosphorylation of individual STAT proteins following the binding of a cytokine to its receptor are regulated in a cell type-restricted fashion. The finding that Jak2 is activated in response to IL-2 uniquely in NK cells suggests that this specificity is mediated at the level of the receptor-kinase complex. Investigation into how these events are regulated will help define the mechanism by which cytokines exert distinct functional effects in different cell types.

Acknowledgments

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References