Published in final edited form as:

Biochem Biophys Res Commun. 2007 March 16; 354(3): 825–829.

Cell type-specific roles of Jak3 in IL-2-induced proliferative signal transduction

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Abstract

Binding of IL-2 to its specific receptor induces activation of two members of Jak family protein tyrosine kinases, Jak1 and Jak3. An IL-2R-reconstituted NIH 3T3 fibroblast cell line proliferates in response to IL-2 only when hematopoietic lineage-specific Jak3 is ectopically expressed. However, the mechanism of Jak3-dependent proliferation in the fibroblast cell line is not known. Here, I showed that Jak3 expression is dispensable for IL-2-induced activation of Jak1 and Stat proteins and expression of nuclear proto-oncogenes in the IL-2R-reconstituted fibroblast cell line. However, Jak3 expression markedly enhanced these IL-2-induced signaling events. In contrast, Jak3 expression was essential for induction of *cyclin* genes involved in the G1-S transition. These data suggest a critical role of Jak3 in IL-2 signaling in the fibroblast cell line and may provide further insight into the cell type-specific mechanism of cytokine signaling.

Keywords

IL-2; fibroblast; Jak kinase; Stat protein; proto-oncogene; Cyclin

Introduction

The high-affinity interleukin-2 (IL-2) receptor (IL-2R) is composed of three subunits; the IL-2R α , IL-2R β , and common cytokine receptor γ (γ c) chains [1-5]. Two members of the Jak kinase family protein tyrosine kinases, Jak1 and Jak3, are associated with IL-2R β and γ c, respectively, and activated upon IL-2 stimulation [6-8]. Activation of Jak1 and Jak3 induces activation of Stat5/Stat3 and other signaling pathways to initiate cell proliferation [9-11]. Although IL-2 can transmit signals in IL-2R-reconstituted fibroblast cell lines [12,13], they do not proliferate in response to IL-2. However, IL-2-induced cell cycle progression was achieved by the additional ectopic expression of Jak3 in NIH 3T3-derived 3T3 $\alpha\beta\gamma$ cells expressing the reconstituted IL-2R (and endogenous Jak1) [6]. It is not shown how ectopic expression of Jak3 supports IL-2-induced proliferation in the fibroblast cell line.

Here, I showed that IL-2 induces activation of Jak1 and Stat proteins and expression of nuclear proto-oncogenes in the absence of Jak3 in 3T3 $\alpha\beta\gamma$. On the other hand, Jak3 expression markedly enhanced IL-2-induced activation of Jak1 and Stat proteins and induction of protooncogenes in this cell line. In contrast, Jak3 expression was indispensable for induction of *cyclin* genes involved in the G1-S transition. These data suggest a critical role of Jak3 in

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IL-2 signaling in the fibroblast cell line and may provide further insight into the cell type-specific mechanism of cytokine signaling.

Materials and Methods

Cell lines

3T3 $\alpha\beta$ is an NIH 3T3-derived cell line ectopically expressing the human IL-2R α and IL-2R β chains [12]. 3T3 $\alpha\beta\gamma$ was derived from 3T3 $\alpha\beta$ and expresses human γc ectopically [12]. J3 was derived from 3T3 $\alpha\beta\gamma$ and expresses mouse Jak3 ectopically [6]. These cell lines were maintained in Dulbecco Modified Eagle's Medium supplemented with 10% (v/v) fetal calf serum (FCS).

Immunoprecipitation and immunoblot Analysis

FCS-depleted cells were unstimulated or stimulated with human IL-2 (10 nM) or mouse IFN γ (25 U/ml) for 10 min. Preparation of whole cell lysates, immunoprecipitation, and immunoblot analyses were done as described previously [10,14-18].

Electrophoretic mobility shift assay (EMSA)

FCS-depleted cells were unstimulated or stimulated with human IL-2 (10 nM) or mouse IFN γ (25 U/ml) for 10 min. Preparation of whole cell lysates and EMSA were performed as described previously [10,14].

Northern blot analysis

FCS-depleted cells were unstimulated or stimulated with human IL-2 (10 nM). At indicated times, cells were harvested and total RNA (10 μ g) were analyzed by Northern blot analysis as previously described [12,19].

Results and Discussion

Ectopic expression of Jak3 enhances IL-2-induced tyrosine phosphorylation of Jak1 in an IL-2R-reconstituted NIH 3T3-derived cell line

3T3 $\alpha\beta\gamma$ is derived from NIH 3T3 fibroblast cell line and has the reconstituted high affinity IL-2R [12]. As shown in Fig. 1A, 3T3 $\alpha\beta\gamma$ cells did not express Jak3 [6], whose expression is detected only in the hematopoietic cell lineages [20,21]. In contrast, this cell line expressed Jak1 (Fig. 1A), whose expression is observed ubiquitously [22]. 3T3 $\alpha\beta\gamma$ does not proliferate in response to IL-2 stimulation [6]. Interestingly, IL-2-induced cell cycle progression was achieved in 3T3 $\alpha\beta\gamma$ -derived J3 cells, in which Jak3 is ectopically expressed by DNA transfection [6]. In J3 cells, IL-2 stimulation induced rapid tyrosine-phosphorylation of Jak3 as expected (Fig. 1B). To elucidate how ectopic expression of Jak3 supports IL-2-induced proliferation in 3T3 $\alpha\beta\gamma$, I first examined tyrosine-phosphorylation of Jak1. It has been shown that activation of Jak1 is well correlated with its tyrosine-phosphorylation levels [23]. As shown in Fig. 1C, IL-2 stimulation induced tyrosine-phosphorylation of Jak1 in 3T3 $\alpha\beta\gamma$. Since IL-2 failed to induce tyrosine-phosphorylation of Jak1 in 3T3 $\alpha\beta$ expressing IL-2R α and IL-2R β but not γc , tyrosine-phosphorylation of Jak1 by IL-2 was mediated by the reconstituted heterotrimeric IL-2R. These results showed that Jak1 could be activated in the absence of Jak3 in 3T3 $\alpha\beta\gamma$. This result is in clear contrast with data from a transformed B cell line derived from a Jak3-deficient SCID patient, in which IL-2-induced Jak1 activation was not observed [24]. It is possible that activation of Jak1 in the B cell line was under the threshold of sensitivity of the experimental condition. Alternatively, it is possible that dependence of Jak1 activation on Jak3 is differentially regulated in different cell types.

Ectopic expression of Jak3 markedly enhanced IL-2-induced tyrosine-phosphorylation of Jak1 (J3 cells, Fig. 1C). This result further confirmed the critical importance of Jak3 for maximal activation of Jak1 and the concept of cross-phosphorylation/activation among receptor-associated Jak kinases [1-5,23].

Ectopic expression of Jak3 enhanced IL-2-induced Stat activation in the IL-2R-reconstituted NIH 3T3-derived cell line

Next, I examined activation of Stat proteins in the IL-2R-reconstituted NIH 3T3-derived cell lines by EMSA using the interferon regulatory factor-1 (IRF-1) interferon- γ (IFN γ) activated site (GAS) probe. As shown in Fig. 2A, IL-2 stimulation induced rapid DNA-binding activities in 3T3 $\alpha\beta\gamma$. This result suggests that the basal activation of Jak1 can mediate IL-2-induced Stat activation in the absence of Jak3 in this cell line. In J3 cells, IL-2 stimulation induced appearance of two distinct DNA-binding activities. The size of the upper band corresponded to that observed in 3T3 $\alpha\beta\gamma$, and its intensity was markedly increased in J3 cells. The lower band was not detected in 3T3 $\alpha\beta\gamma$. As shown in Fig. 2B, the upper DNA-binding complexes contained Stat3 and Stat5, whereas the lower one contained Stat1. These results indicate that Jak1 activation can induce activation of Stat5/Stat3 and Jak3 enhances IL-2-mediated Stat5/Stat3 activation in the NIH 3T3-derived cells. Interestingly, Stat1 activation by IL-2 was totally dependent on expression of Jak3. It has been reported that IL-2 can activate Stat1 in addition to Stat5/Stat3 and Stat1 activation was only observed in the presence of high concentrations of IL-2 [9]. This finding is consistent with my observation that Stat1 activation is detected only when activation of Jak3 and maximal activation of Jak1 are achieved by ectopic expression of Jak3 in 3T3 $\alpha\beta\gamma$.

Ectopic expression of Jak3 enhances IL-2-induced nuclear proto-oncogene expression in the IL-2R-reconstituted NIH 3T3-derived cell line

It has been shown that induction of nuclear proto-oncogenes by growth-promoting cytokines plays important roles in cell proliferation [19,25]. IL-2 stimulation induces expression of protooncogenes including *c-fos* and *c-myc* in 3T3 $\alpha\beta\gamma$ (Fig. 3A) as previously described [12]. It is likely that IL-2-induced activation of Jak1 and Stat5/Stat3 mediates the proto-oncogene induction in 3T3 $\alpha\beta\gamma$. I examined how IL-2-induced proto-oncogene expression is affected by ectopic expression of Jak3 by Northern blot analysis. As shown in Fig. 3A, induction of *c-myc* and *c-fos* was significantly enhanced by ectopic expression of Jak3. Furthermore, *c-myc* induction was sustained in J3 cells (Fig. 3B). These results showed that although Jak3 is dispensable for proto-oncogene expression in 3T3 $\alpha\beta\gamma$, it enhances expression of nuclear protooncogenes by IL-2. These results also suggest that quantitative regulation of these protooncogenes is critical for cell proliferation in NIH 3T3-derived cells.

Essential role of Jak3 in the induction of *cyclin* genes in IL-2R-reconstituted NIH 3T3-derived cell lines

Cyclins play essential roles in cell cycle progression, and their expression is induced by growth-promoting stimuli [26]. Among others, D-type Cyclins and Cyclin E have been shown to be critical for the G1-S transition [27]. To examine how *cyclin* mRNA induction is affected by ectopic expression of Jak3 in IL-2R-reconstituted NIH 3T3-derived cells, I performed Northern blot analysis using specific cDNA probes. In 3T3 $\alpha\beta\gamma$, IL-2 stimulation marginally upregulated expression of *cyclin D1*, *D2*, *D3*, and *E* genes (Fig. 4). In contrast, expression of these *cyclin* genes was significantly induced by IL-2 in J3 cells, indicating an essential role of Jak3 in the induction of the *cyclin* genes.

In conclusion, I showed that IL-2 induces activation of Jak1 in the absence of Jak3 in an IL-2R-reconstituted NIH 3T3-derived cell line, 3T3 $\alpha\beta\gamma$ (Fig. 1). This result was unexpected because Jak3 is indispensable for IL-2-induced Jak1 activation in human B cells [24]. It is an interesting

future issue how Jak1 activation is regulated in a cell type-specific manner. In 3T3 $\alpha\beta\gamma$, Stat5/Stat3 activation and induction of proto-oncogenes were observed in the absence of Jak3 expression (Fig. 2 and 3). In contrast, IL-2-induced Stat1 activation was dependent on expression of Jak3 (Fig. 2). Although IL-2-induced activation of Jak1 and Stat5/Stat3 as well as induction of proto-oncogenes were observed in 3T3 $\alpha\beta\gamma$, IL-2 stimulation failed to induce the G1-S *cyclin* genes (Fig. 4) and the S-phase transition [6,28]. Jak3 expression augmented activation of Jak1 and Stat5/Stat3 and induction levels of nuclear proto-oncogenes (Fig. 1-3). Furthermore, induction of the G1-S *cyclin* genes and cell cycle progression were observed in J3 cells but not in 3T3 $\alpha\beta\gamma$ (Fig. 4) [6,28]. These results suggest the importance of enhanced signaling by Jak3 expression for cell cycle progression. It is also suggested that cell cycle progression requires signaling strength above a certain threshold. Alternatively, it cannot be excluded at this stage whether Jak3 transmits signaling distinct from that induced by Jak1 activation. This is an important future issue.

Acknowledgements

I thank Dr. T. Taniguchi for IL-2R-reconstituted cell lines. This work is supported by NIH grant R01 AI059315 (H. F.).

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Figure 1.

Jak3 enhances activation of Jak1 in an IL-2R-reconstituted NIH 3T3-derived cell line. (A) Expression of Jak1 and Jak3 in 3T3 $\alpha\beta\gamma$ and J3. Cell lysates of these cells were separated by 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted with anti-Jak1 or anti-Jak3 antibody (Ab). (B) Activation of Jak3 by IL-2 in J3 cells. FCS-depleted J3 cells were unstimulated or stimulated with human IL-2 (10 nM) for 10 min. Cells were lysed, and Jak3 was immunoprecipitated with anti-Jak3 Ab. Immunoprecipitates were separated by SDS-PAGE and subjected to immunoblot analysis with anti-phosphotyrosine (anti-PY) Ab (upper panel). Jak3 protein levels were determined by immunoblot analysis with anti-Jak3 Ab (lower panel). (C) Expression of Jak3 enhances activation of Jak1. FCS-depleted 3T3 $\alpha\beta$, 3T3 $\alpha\beta\gamma$ and J3 were unstimulated or stimulated with human IL-2 (10 nM) or mouse IFN γ (25 U/ml) for 10 min. Cells were lysed, and Jak1 was immunoprecipitated with anti-Jak1 Ab. Immunoprecipitates were separated by SDS-PAGE and subjected to immunoblot analysis with anti-PY Ab (upper panel). Jak1 protein levels were determined by immunoblot analysis with anti-Jak1 Ab (lower panel).

Figure 2.

Jak3 enhances activation of Stat proteins in the IL-2R-reconstituted NIH 3T3-derived cell line. (A) 3T3 $\alpha\beta\gamma$ and J3 were mock-stimulated or stimulated with human IL-2 (10 nM) or mouse IFN γ (25 U/ml) for 10 min. Whole cell lysates were incubated with ^{32}P -labeled IRF-1 GAS probe. Complexes were resolved on 4% acrylamide gels and detected by autoradiography. The upper arrow indicates the position of DNA-binding complex detected in IL-2-stimulated 3T3 $\alpha\beta\gamma$ and J3. The lower arrow indicates the position of DNA-binding complex detected in IL-2-stimulated J3 and IFN γ -stimulated cells. (B) Supershifts of IL-2-induced DNA-binding complex. Whole cell lysates were preincubated for 1 hr at 4°C with non-immune serum (NIS) or Abs specific to Stat1, Stat3, or Stat5. The upper arrow indicates the position of DNA-binding complex containing Stat3 and Stat5. The lower arrow indicates the position of DNA-binding complex containing Stat1.

Figure 3.

Enhanced proto-oncogene induction by Jak3 expression. (A) Enhanced IL-2-induced expression of *c-fos* and *c-myc* by ectopic expression of Jak3. FCS-depleted cells were unstimulated or stimulated with human IL-2 (10 nM). At indicated times, cells were harvested and total RNA (10 μ g) were analyzed by Northern blot analysis. The filters were stained with methylene blue to show total RNA (18S ribosomal RNA). (B) Sustained IL-2-induced expression of *c-myc* mRNA in J3. FCS-depleted cells were unstimulated or stimulated with human IL-2 (10 nM). At indicated times, cells were harvested and total RNA (10 μ g) were analyzed by Northern blot analysis. The filters were stained with methylene blue to show total RNA (28S ribosomal RNA).

Figure 4.

IL-2-induced expression of the G1-S *cyclin* genes by extopic expression of Jak3. FCS-depleted cells were unstimulated or stimulated with human IL-2 (10 nM). At indicated times, cells were harvested and total RNA (10 μ g) were analyzed by Northern blot analysis. The filters were stained with methylene blue to show total RNA (18S ribosomal RNA).