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Interleukin 2-induced activation of JAK3: possible involvement in signal transduction for c-*myc* induction and cell proliferation

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Abstract We have investigated the role of JAK3 in interleukin 2 (IL-2)-induced signal transduction with a human T cell line, ED40515(−), lacking expression of the IL-2 receptor γ chain and its sublines transfected with wild-type or mutant cDNAs of the IL-2 receptor γ chain. Our results demonstrated that the membrane-proximal cytoplasmic region, encompassing the src homology region 2 (SH2)-like subdomain, of the γ chain is essential for association and activation of JAK3. Furthermore, IL-2-induced activation of JAK3 paralleled induction of the c-*myc* gene and DNA synthesis but not induction of the c-*fos* and c-*jun* genes. These results support the hypothesis that JAK3 plays a pivotal role in the IL-2 receptor-mediated signals for cell growth.

Key words: IL-2; IL-2Rγ chain; JAK3; Signal transduction

1. Introduction

The functional IL-2 receptor (IL-2R) is known to be a heterotrimer composed of the α, β and γ chains or a heterodimer of the β and γ chains, indicating that the β and γ chains are essential for formation of the functional IL-2R [1]. The β and γ chains belong to the cytokine receptor family which has no catalytic domain like kinases. IL-2 induced, however, tyrosine phosphorylation of the β and γ chains of IL-2R [2,3] and of cellular proteins [4–8], and the IL-2R complex contained tyrosine kinase activity [3,9,10].

During studies to identify tyrosine kinase(s) associated with the IL-2R complex, src type tyrosine kinases, Lck, Lyn and Fyn, were demonstrated to be activated by IL-2 stimulation [11–13], and, in particular, Lck was shown to bind directly to the so-called acidic region of the β chain [14]. However, it is not yet determined whether such src type tyrosine kinases are involved in IL-2-induced cell growth promotion, because a β chain mutant lacking the acidic region, which does not associate with Lck, still retains the ability to transduce cell growth signal in an IL-2-dependent manner [15,16].

There is accumulating evidence that cytoplasmic tyrosine kinase of JAK-Tyk family, such as JAK1, JAK2 and Tyk2, are involved in signal transduction mediated by some cytokines including interferon (IFN) α/β and γ [17–21], erythropoietin (EPO) [22], growth hormone (GH) [23], interleukin 3 (IL-3) [24], interleukin 6 (IL-6), ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM) [25,26] and granulocyte-colony-stimulating factor (G-CSF) [27]. In this context, we have asked whether the JAK-Tyk family is associated with the IL-2/IL-2R system, and recently found that JAK1 and 114 kDa JAK2-related kinases are physically associated with the β chain and the γ chain, respectively, and at least the 114 kD JAK2-related kinase is activated by IL-2 stimulation [28]. Very recently a novel member of JAK-Tyk family, JAK3 or L-JAK was cloned [29,30]. The present study using anti-JAK3 antibody demonstrated that the 114 kDa JAK2-related kinase associated with the γ chain of IL-2R is identical to JAK3, and further demonstrated the significant roles of JAK3 in IL-2-mediated signals for induction of the c-*myc* gene and DNA synthesis.

2. Materials and methods

2.1. Plasmid construction

Expression vector plasmids used are pSRG1 containing the wild-type γ chain cDNA of IL-2R, pSRGdC30 and pSRGdC68 containing γdC30 and γdC68 mutant γ chain cDNA deleted of the C-terminal 30 and 68 amino acids, respectively [31]. pSRGdSH contains γdSH mutant γ chain cDNA deleted of the SH2-like subdomain. For the deletion of the SH2-like subdomain of the γ chain, the 136-bp *Eco*RI–*Hin*fI fragment was removed from pSRG1 and the resultant plasmid vector was ligated after T4 DNA polymerase treatment.

2.2. Cell lines

ED40515(−), previously referred to as ED515-I, is a subclone of ED40515, an HTLV-I transformed human T cell line [32]. ED40515(−) expresses the α and β chains of IL-2R but not the IL-2R γ chain and proliferates independently of IL-2 [33]. EDγ cell lines (EDγ-2, -15 and -16) were independently established from ED40515(−) by transfection with a vector plasmid, pSRG1. EDγdC30, EDγdC68 and EDγdSH cell lines (EDγdC30-1, -2 and -4, EDγdC68-3, -5 and -7 and EDγdSH-1, -6 and -10) were similarly established from ED40515(−) by stable transfection with pSRGdC30, pSRGdC68 and pSRGdSH plasmids. All the cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) and antibiotics.

2.3. Antibodies

TU11 and TUGh4 mAbs are specific for the human IL-2Rβ chain [34] and human IL-2Rγ chain [35], both of which do not interfere with IL-2 binding to IL-2R. PAR3 mAb against human parvovirus B19 was used as a control [36]. Anti-JAK1 and anti-JAK2 antibodies specific for murine JAK1 and JAK2, which are cross-reactive to human JAK1 and JAK2, respectively, and 4G10 mAb specific for phosphotyrosine were purchased from UBI (Lake Placid, NY). Anti-rat JAK3 antibody was

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Abbreviations: IL-2, interleukin 2; IL-2R, interleukin-2 receptor; mAb, monoclonal antibody; SH2, src homology region 2.

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obtained with immunization of rat JAK3 synthetic peptide (Asp^{169}–Gly^{182}).

2.4. [^{3}H]Thymidine incorporation assay

Short-term cell proliferation in response to IL-2 were measured by [^{3}H]thymidine incorporation assays. Cells (2×10^4) were cultured in 200 μl of medium containing 0.5% FCS in the presence of IL-2 at indicated concentrations for 72 h in triplicate. [^{3}H]Thymidine was added at 1.0 μCi per well 4 h before termination of incubation. The incorporated [^{3}H]thymidine was counted with a liquid scintillation counter.

2.5. Northern (RNA) blotting and probes

Total cellular RNA was isolated by extraction with guanidium thiocyanate from cells stimulated with 10 nM IL-2. Northern blot hybridization and probes were described previously [31].

2.6. Immunoblotting

Cells were incubated for 5 min in the presence or absence of 10 nM of IL-2, and then lysed with NP-40 cell extraction buffer or digitonin cell extraction buffer (1% digitonin, 150 mM NaCl, 10 mM triethanolamine, 10 mM iodoacetamide, 1 mM EDTA and 20 μg/ml aprotinin). Cell lysates were immunoprecipitated with indicated antibodies. The immunoprecipitates were separated by SDS-PAGE and transferred to PVDF filters (Millipore, Japan). After incubation in PBS containing 2% BSA, the filters were then incubated with indicated antibodies, followed by incubation with ^{125}I-labeled protein A (ICN, Costa Mesa, CA).

2.7. In vitro immunecomplex kinase assay

The immunoprecipitates were suspended in the kinase reaction buffer [10 mM HEPES (pH 7.5), 50 mM NaCl, 0.1 mM Na_3VO_4, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 0.5 mM DTT, 0.1% Triton X-100]. The kinase reaction was started by addition of 10 μCi of [γ^{32}P]ATP (> 5000 Ci/mmol, Amersham) and continued for 10 min at 22°C. The reaction was terminated by addition of 20 μl of SDS gel loading buffer.

3. Results and discussion

3.1. Effects of mutant γ chains on IL-2-mediated signal transduction

A human T cell line, ED40515(−), expresses the IL-2R α and β chains, but is undetectable in expression of the γ chain at either protein or mRNA level ([33], our unpublished data). The wild-type γ chain and its cytoplasmic deletion mutants were stably introduced into ED40515(−) cells by electroporation. The mutants are γdC30 lacking C-terminal 30 amino acids, γdC68 lacking the C-terminal 68 amino acids and γdSH lacking the SH2-like subdomain. The SH2-like subdomain is intact in γdC30, but it is abolished in γdC68. We picked up representatives of the ED40515(−) transfectants with the wild-type, γdC30, γdC68 and γdSH, and renamed EDγ, EDγdC30, EDγdC68 and EDγdSH, respectively. Expression of the transfected γ chains on the clones was confirmed by immunostaining and immunoprecipitation with TUGh4, a monoclonal antibody (mAb) specific for the human IL-2Rγ chain (data not shown).

We examined the effects of IL-2 on growth of the transfectants. Incorporation of [^{3}H]thymidine was monitored by using three independent clones of each transfectant with the wild-type or mutant γ chains. Cells (2×10^4 cells/well) in microplates were stimulated with indicated doses of IL-2 and labeled with [^{3}H]thymidine for 4 h. IL-2 induced [^{3}H]thymidine incorporation in three clones, EDγ-2, -15 and -16, of the wild-type γ chain transfectant in an IL-2-dose-dependent manner. Similar incorporation was seen with three clones, EDγdC30-1, -2, and -4, of the transfectant with γdC30, however no increase in [^{3}H]thymidine incorporation was observed with EDγdC68-3, -5 and -7

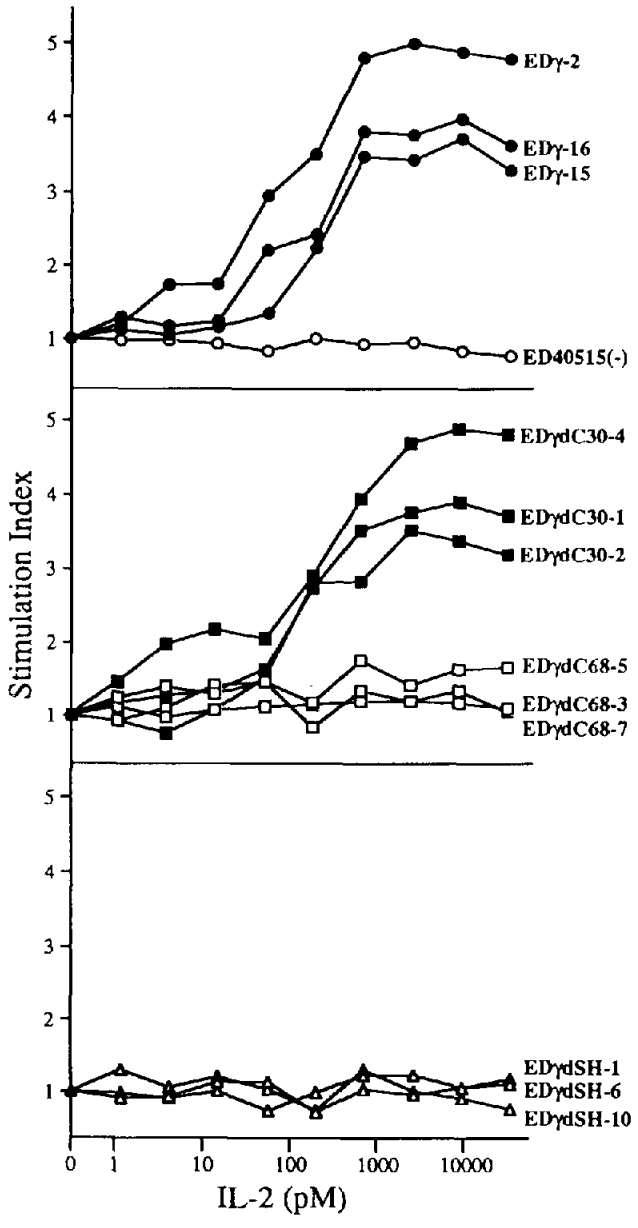

Fig. 1. [^{3}H]Thymidine incorporation assay. Cells (2×10^4) were cultured at various concentrations of IL-2 and measured for incorporation of [^{3}H]thymidine in triplicate. Stimulation indexes represent the ratios of radioactivity of incorporated [^{3}H]thymidine in the presence of IL-2 to that in the absence of IL-2. ED40515(−), EDγ-2, -15 and -16, EDγdC30-1, -2 and -4, EDγdC68-3, -5 and -7, EDγdSH-1, -6 and -10 in medium containing 0.5% FCS without IL-2 gave [^{3}H]thymidine incorporation of 39,710 ± 3,258, 30,430 ± 743, 42,035 ± 1,395, 33,611 ± 929, 21,405 ± 1,151, 39,787 ± 1,424, 45,682 ± 857, 24,390 ± 1,330, 34,699 ± 3,052, 24,930 ± 2,070, 28,58 ± 2,220, 26,604 ± 3,018 and 30,336 ± 3,704 cpm ± S.E.M., respectively. Each plot represents the mean value ± S.E.M. of incorporated [^{3}H]thymidine in triplicate.

transfectants and EDγdSH- 1, -6 and -10 transfectants as well as in ED40515(−) cells (Fig. 1).

The transfectants were further investigated for their expression of nuclear proto-oncogenes, c-*fos*, c-*jun* and c-*myc*, which is another parameter of IL-2-dependent signals. Transfectant cells were cultured in the presence of 10 nM IL-2. Total RNA was extracted from those cells and subjected to Northern blot

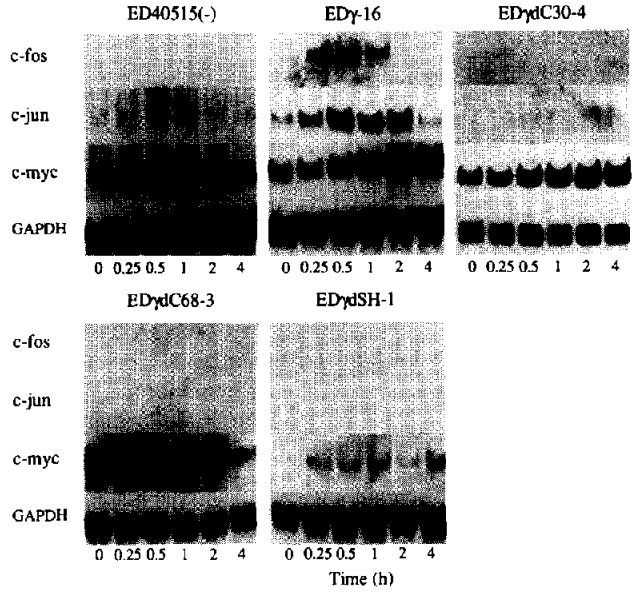

Fig. 2. IL-2-dependent mRNA expression of nuclear proto-oncogenes in the transfectants. Cells were stimulated with 10 nM IL-2 for the indicated times and harvested for RNA preparation. 30 μg of total RNAs were loaded on agarose gel electrophoresis. After blotting to a nylon filter, the filter was incubated with indicated ^{32}P-labeled probes. The relative amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was monitored to check the amount of total RNA applied to each lane.

analysis with appropriate probes (Fig. 2). mRNA for the c-*fos* and c-*jun* genes in EDγ-16 cells was induced by IL-2 stimulation, and expression of mRNA for the c-*myc* gene was enhanced, similar to the mouse fibroblastoid L929 cell transfectant reconstituted with the high-affinity IL-2 receptor by transfection with the wild α, β and γ chains [31]. No effect of IL-2 was seen with EDγdC68-3 EDγdSH-1 and ED40515(−) cell lines. Interestingly, IL- 2-dependent enhancement of c-*myc* mRNA was segregated from induction of c-*fos* and c-*jun* mRNA in EDγdC30-4 cells.

These results indicate that the membrane-proximal cytoplasmic region including the SH2-like subdomain of the γ chain is required for IL-2-mediated signal transduction for cell growth and enhancement of the c-*myc* gene, whereas the region of the C-terminal 30 amino acids of the γ chain is essential for induction of the c-*fos* and c-*jun* genes but not for enhancement of the c-*myc* gene and cell growth.

3.2. *Association of JAK1 and the 114 kD JAK2-related molecule with the β and γ chains*

Transfectants were lysed with digitonin lysis buffer, and the β and γ chains were then immunoprecipitated with TU11 and TUGh4, respectively. As controls, ED40515(−) cells were immunoprecipitated with a control mAb, PAR3, and with anti-JAK1 or anti-JAK2 antibody. The immunoprecipitates werre separated by SDS-PAGE and transferred to filters which were then immunoblotted with anti-JAK2 antibody. A band with an apparent molecular mass of 114 kDa was detected in the TUGh4-directed immunoprecipitates from EDγ-16 and EDγdC30-4 (Fig. 3, upper panel, lanes 2 and 3). No band was associated with the immunoprecipitates from EDγdC68-3 and EDγdSH-1, and with β chains which were immunoprecipitated with the TU11 from all cell lines. These results demonstrated that the 114 kDa JAK2-related molecule directly or indirectly links to the membrane-proximal cytoplasmic region, encompassing the SH2-like subdomain, of the γ chain. As expected, the 114 kD JAK2-related molecule was not detected in the immunoprecipitates by a control antibody, PAR3, and an anti-JAK1 antibody.

The procedure of immunoblot with anti-JAK2 antibody detected two major bands (120 kDa and 97 kDa) and two minor bands (114 kDa and 102 kDa) with the anti-JAK2 antibody-directed immunoprecipitate using lysates from ED40515(−) (Fig. 3, lane 13). All four bands were eliminated by pretreatment with the synthetic oligopeptides from murine JAK2, which were immunized to generate anti-JAK2 antibody used in this study (data not shown), indicating that the four molecules are specifically precipitated by anti-JAK2 antibody. Among them only the minor 114 kDa JAK2-related molecule seemed to be associated with the γ chain.

We have recently found that the β chain is associated with JAK1 [28]. Thus, association of JAK1 with the β chain was examined with the transfectants bearing the wild-type and mutant γ-chains (Fig. 3, lower panel). The band corresponding to JAK1 was seen with the TU11- directed immunoprecipitates from all the transfectants, irrespective of co-expression of the γ chains.

3.3. *In vivo tyrosine phosphorylation of JAK3 in response to IL-2*

We previously reported that JAK1 and the 114 kDa JAK2-related molecules were tyrosine phosphorylated with IL-2 stim-

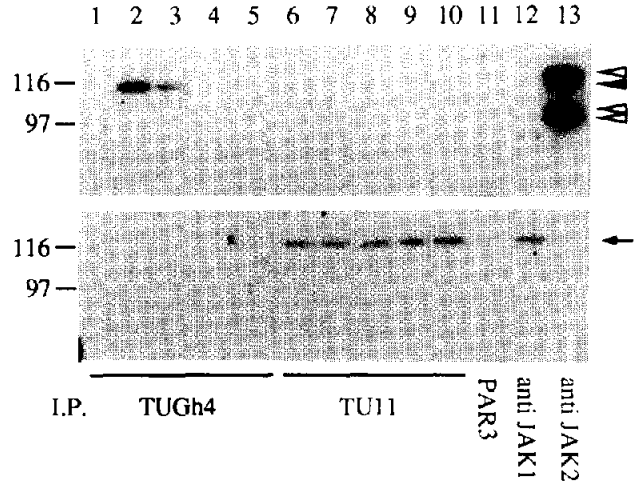

Fig. 3. Physical association of JAK1 and the 114 kD JAK2-related molecule with the IL-2R. Cells (1×10^8 for lanes 1–11, 1×10^7 for lanes 12 and 13) were lysed with digitonin cell extraction buffer. The γ chain (lanes 1–5) and the β chain (lanes 6–10) were immunoprecipitated from cell lysates of ED40515(−) (lanes 1 and 6), EDγ-16 (lanes 2 and 7), EDγdC30-4 (lanes 3 and 8), EDγdC68-3 (lanes 4 and 9) and EDγdSH-1 (lanes 5 and 10). For controls, immunoprecipitation was performed with PAR3 mAb (lane 11), anti-JAK1 antibody (lane 12) and anti-JAK2 antibody (lane 13) from cell lysate of ED40515(−). The immunoprecipitates were separated by SDS-PAGE and blotted to a filter. The filter was then incubated with anti-JAK2 antibody (upper panel), followed by incubation with ^{125}I-labeled protein A. After stripping, the same filter was similarly incubated with anti-JAK1 antibody and then with ^{125}I-labeled protein A (lower panel). Molecular sizes of standards ($M_r \times 10^{-3}$) are indicated and the 114 kDa JAK2-related molecule and JAK1 are also shown by a closed arrowhead and an arrow, respectively. Other JAK2-related molecules are indicated by open arrowheads.

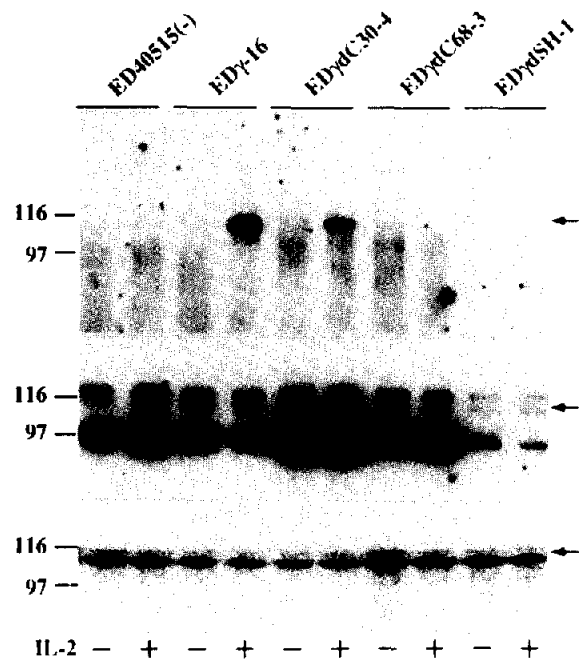

Fig. 4. IL-2-induced tyrosine phosphorylation of JAK3 in the transfectants. Cells were stimulated with 10 nM IL-2 for 5 min, lysed with the NP-40 cell extraction buffer and immunoprecipitated with anti-JAK2 antibody. The immunoprecipitates were separated by SDS-PAGE and blotted to a filter. The filter was incubated with anti-phosphotyrosine antibody and then with ^{125}I-labeled protein A (upper panel). After stripping, the same filter was similarly incubated with anti-JAK2 antibody and ^{125}I-labeled protein A (middle panel). After stripping, the same filter was similarly incubated with anti-rat JAK3 antibody and ^{125}I-labeled protein A (lower panel). Molecular sizes of standards ($M_r \times 10^{-3}$) are indicated and the 114 kDa JAK2-related molecule (upper and middle panel) and JAK3 (lower panel) are shown by arrows.

ulation on human T cell lines and phytohemagglutinin-stimulated human peripheral blood leukocyte [28]. Then we examined the effects of IL-2 on phosphorylation of the 114 kDa JAK2-related molecule on the transfectants which express the mutant γ chains. The 114 kDa JAK2-related molecules were immunoprecipitated with anti-JAK2 antibody, from lysates of the transfectants before and after IL-2 stimulation. The immunoprecipitates were separated by SDS-PAGE and immunoblotted with anti-phosphotyrosine antibody. IL-2 induced tyrosine phosphorylation of the 114 kDa JAK2- related molecule in EDγ-16 and EDγdC30-4 cells but not in EDγdC68-3 and EDγdSH-1 cells as well as ED40515(−) (Fig. 4, upper panel). The same membrane was probed with anti-JAK2 antibody after stripping the anti-phosphotyrosine antibody (Fig. 4, middle panel). Four bands (120, 114, 102 and 97 kDa) of JAK2-related molecules were seen with all the cell lines. Among the four bands, the second slowly migrating band (indicated by an arrow) was phosphorylated. Thus, again, only the 114 kDa JAK2-related molecule was shown to be tyrosine-phosphorylated upon IL-2 stimulation. Very recently human L-JAK/JAK3 was cloned [30], and we noticed that the amino acid sequence of synthetic peptide for immunization of mouse JAK2 (Asp^{758}–Lys^{776}) was very similar to that of L-JAK/JAK3 (Asp^{730}–Lys^{748}). 14 amino acids among the JAK2 synthetic peptide are identical to that of JAK3. So we examined if the 114 kDa JAK2-related molecule is identical to the JAK3 or not. The same membrane was probed with anti-rat JAK3 antibodies after stripping the anti-JAK2 antibody. The amino acid sequence of synthetic peptide of rat JAK3, which was used for immunization of rat JAK3, was almost similar to that of human JAK3 (13 amino acids of the 14-amino-acid synthetic peptide were identical to human JAK3). As expected, only one band of JAK3 with an apparent molecular mass of 114 kDa was seen with all the cell lines (Fig. 4, lower panel). The band was not detected in the presence of JAK3 synthetic peptide (data not shown). We then concluded that the 114 kDa JAK2- related molecule is identical to the JAK3, indicating that anti-JAK2 antibody used crossreacts to human JAK2 and JAK3. Unfortunately, because the anti-rat JAK3 antibody could not immunoprecipitate the human JAK3, we immunoprecipitated the JAK3 with the anti- JAK2 antibody in the following study.

3.4. *In vitro phosphorylation of JAK3*

We assumed that tyrosine phosphorylation of JAK3 in response to IL-2 reflects activation of JAK3 kinase. The possibility was examined by the in vitro kinase assay. The transfectants were precipitated with anti- JAK2 antibody before and after IL-2 stimulation, and the immunoprecipitates were employed for the in vitro kinase assay with [γ^{32}P]ATP. IL-2 significantly affected phosphorylation of the JAK3 molecule from EDγ-16 and EDγdC30-4 cells, but not from other transfectants and the parental ED40515(−) cells (Fig. 5A). Phosphoamino acid analysis of the bands clearly indicated that in vitro phosphorylation of JAK3 after IL-2 stimulation was prominent on tyrosine residues (Fig. 5B). Similarly, JAK1 kinase activation was examined, but it was undetectable in any of the transfectants tested (data not shown).

Our results clearly demonstrated that the cytoplasmic SH2-like subdomain of the γ chain, which is essential for IL-2-mediated signaling for the induction of the c-*myc* gene and DNA synthesis, is required for association and activation of JAK3, while the region containing the C- terminal 30 amino acids of the γ chain, which is essential for induction of the c-*fos* and c-*jun* genes but not induction of the c-*myc* gene and DNA synthesis, has no effect on the activation and association of JAK3 with the γ chain.

The two distinct signaling pathways from the IL-2 receptor, one for induction of the c-*fos* and c-*jun* genes and the other for induction of the c-*myc* gene that correlates with cell growth promotion, were previously observed in transfectants with the β and γ chains [31,37]. The IL-2-mediated induction of the c-*fos* and c-*jun* genes has been suggested to be accompanied with activation of the Src type tyrosine kinases such as Lck, Fyn and Lyn, and activation of Ras and Raf-1 [16,38–40]. The acidic region of the β chain was found to be necessary for association of these src type tyrosine kinases [14,16]. However, IL-2-induced tyrosine phosphorylation of cellular proteins and cell proliferation were observed in transfectant cell lines expressing the α and γ chains and the β chain mutant lacking the acidic region (Ohbo et al., unpublished data). These observations suggested that other tyrosine kinase(s) rather than the src type tyrosine kinases may participate in IL-2-mediated cell growth signal transduction.

Intriguingly, the present study demonstrated that JAK3 association with the γ chain and activation of JAK3 can be separated from the IL-2-mediated signal transduction pathway for induction of the c-*fos* and c-*jun* genes, rather association and

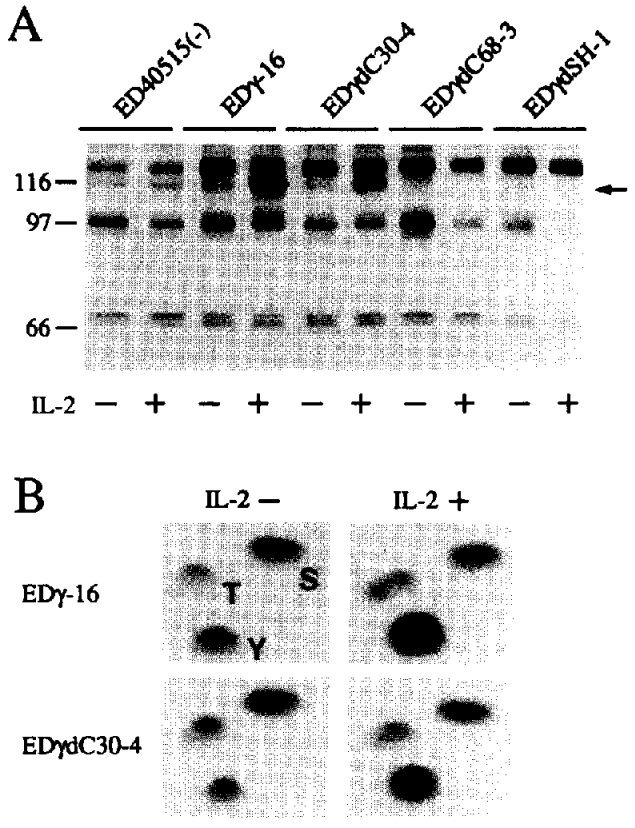

Fig. 5. In vitro phosphorylation of JAK3. Cells were stimulated with 10 nM IL-2 for 5 min, lysed with the NP-40 cell extraction buffer and immunoprecipitated with anti-JAK2 antibody. The immunoprecipitates were subjected to in vitro immunecomplex kinase assays (A). Molecular sizes of standards ($M_r \times 10^{-3}$) are indicated and JAK3 bands are shown by arrow. Bands corresponding to JAK3 from EDγ-16 and EDγdC30-4 cells before and after IL-2 stimulation was excised from the gel and examined for phosphoamino acids (B). Positions of phosphoserine (S), phosphothreonine (T) and phosphotyrosine (Y) are shown.

activation of JAK3 parallels induction of the c-*myc* gene and DNA synthesis. Thus, JAK3 is suggested to play crucial roles in IL-2- mediated signal transduction for induction of c-*myc* mRNA followed by DNA synthesis.

There is accumulating evidence that JAK-Tyk kinases are tyrosine-phosphorylated upon stimulation with many cytokines. IFNα/β, IFNγ, IL-6, LIF, OSM and CNTF-induced signals involve two distinct members of the JAK-Tyk family. Two members associated with receptors for such cytokines are thought to be essential for signal transduction mediated by ligands. In the case of IL-2, similar to these cytokines, JAK1 and JAK3 link to the β and γ chains, respectively, even in the absence of IL-2. And the previous and the present results suggested that both of these kinases seem to be essential for IL-2-induced cell growth signaling [28].

Abnormality of the γ chain is closely related to X- linked severe combined immunodeficiency (XSCID) characterized by impairment of early T cell development [41]. Mysteriously IL-2-deficient mice did not show XSCID concomitant phenotypes [42]. Recent studies may, however, pave the way to dissolve the discrepancy. We and others demonstrated that the γ chain is shared by receptors for interleukin 4 (IL-4) and interleukin 7 (IL-7) [43–46]. Thus cytokines which recruit the γ chain as a receptor subunit are possible candidates of which dysfunction induces impairment of early T cell development in XSCID. Indeed, IL-4 and IL-7 are shown to have effects on T cells. Collectively, JAK3 is assumed to be involved in signal transduction from the IL-4 receptor and IL-7 receptor, similar to the IL-2 receptor. Our preliminary experiment indicated that IL-4 and IL-7 induce tyrosine phosphorylation of JAK3 (Tanaka et al., unpublished data). So we are very interested in the phenotype of JAK3 knock out mice besides of the γ chain knock out mice. Next questions to be addressed are how specific these receptors including the γ chain and JAK family members transduce signals. Although we have not yet defined signal transducers associated with the α chains of IL-4R and IL-7R, it is also possible that they associate with other members of the JAK-Tyk family, such as JAK1, Tyk2 or other members, like other cytokine receptors mentioned above. The IL-2Rβ chain, IL- 4Rα chain and IL-7Rα chain may transduce specific signals depending on ligands, while the γ chain may generate signals common to ligands. Two signal pathways such as JAK1 and JAK3 in IL-2-mediated signals may interact with each other, resulting in complete ligand-dependent signal transduction. Identification of substrates for IL-2-, IL-4- or IL-7-activated JAK3 will be necessary to see the functional roles of the JAK-Tyk family in lymphokine signal transduction system.

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References

[1] Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., Munakata, H., Nakamura, M. and Sugamura, K. (1992) Science 257, 379–382.
[2] Asao, H., Takeshitaz, T., Nakamura, M., Nagata, K. and Sugamura, K. (1990) J. Exp. Med. 171, 637–644.
[3] Asao, H., Kumaki, S., Takeshita, T., Nakamura, M. and Sugamura, K. (1992) FEBS Lett. 304, 141–145.
[4] Saltzman, E.M., Thom, R.R. and Casnellie, J.E. (1988) J. Biol. Chem. 263, 6956–6959.
[5] Morla, A.O., Schreursx, J., Miyajima, A. and Wang, J.Y.J. (1988) Mol. Cell. Biol. 8, 2214–2218.
[6] Ferris, D.K., Wiillette-Brown, J., Ortaldo, J.R. and Farrar, W.L. (1989) J. Immunol. 143, 870–876.
[7] Sharon, M., Gnarra, J.R. and Leonard, W.J. (1989) J. Immunol. 143, 2530–2533.
[8] Mills, G.B., May, C., McGill, M., Fung, M., Baker, M., Sutherland, R. and Greene, W.C. (1990) J. Biol. Chem. 265, 3561–3567.
[9] Merida, I. and Gaulton, G.N. (1990) J. Biol. Chem. 265, 5690–5694.
[10] Fung, M.R., Scearce, R.M., Hoffman, J.A., Peffer, N.J., Hammes, S.R., Hosking, J.B., Schmandt, R., Kuziel, W.A., Haynes, B.F., Mills, G.B. and Greene, W.C. (1991) J. Immunol. 147, 1253–1260.
[11] Horak, I.D., Gress, R.E., Lucas, P.J., Horak, E.M., Waldmann, T.A. and Bolen, J.B. (1991) Proc. Natl. Acad. Sci. USA 88, 1996–2000.
[12] Torigoe, T., Saragovi, H.U. and Reed, J.C. (1992) Proc. Natl. Acad. Sci. USA 89, 2674–2678.
[13] Kobayashi, N., Kono, T., Hatakeyama, M., Minami, Y., Miyazaki, T., Perlmutter, R.M. and Taniguchi, T. (1993) Proc. Natl. Acad. Sci. USA 90, 4201–4205.

[14] Hatakeyama, M., Kono, T., Kobayashi, N., Kawahara, A., Levin, S.D., Perlmutter, R.M. and Taniguchi, T. (1991) Science 252, 1523–1528.
[15] Hatakeyama, M., Mori, H., Doi, T. and Taniguchi, T. (1989) Cell 59, 837–845.
[16] Taniguchi, T. and Minami, Y. (1993) Cell 73, 5–8.
[17] Velazquez, L., Fellous, M., Stark, G.R. and Pellegrini, S. (1992) Cell 70, 313–322.
[18] Müller, M., Briscoe, J., Laxton, C., Guschin, D., Ziemiecki, A., Silvennoinen, O., Harpur, A.G., Barbieri, G., Witthuhn, B.A., Schindler, C., Pellegrini, S., Wilks, A.F., Ihle, J.N., Stark, G.R. and Kerr, I.M. (1993) Nature 366, 129–135.
[19] Watling, D., Guschi, D., Müller, M., Silvennoinen, O., Witthuhn, B.A., Quelle, F.W., Rogers, N.C., Schindler, C., Stark, G.R., Ihle, J.N. and Kerr, I.M. (1993) Nature 366, 166–170.
[20] Shuai, K., Ziemiecki, A., Wilks, A.F., Harpur, A.G., Sadowski, H.B., Gilman, M.Z. and Darnell, J.E. (1993) Nature 366, 580–583.
[21] Silvennoinen, O., Ihle, J.N., Schlessinger, J. and Levy, D.E. (1993) Nature 366, 583–585.
[22] Witthuhn, B.A., Quelle, F.W., Silvennoinen, O., Yi, T., Tang, B., Miura, O. and Ihle, J.N. (1993) Cell 74, 227–236.
[23] Argetsinger, L.S., Campbell, G.S., Yang, X., Witthuhn, B.A., Silvennoinen, O., Ihle, J.N. and Carter-Su, C. (1993) Cell 74 (237–244.
[24] Silvennoinen, O., Witthuhn, B.A., Quelle, F.W., Cleveland, J.L., Yi, T. and Ihle, J.N. (1993) Proc. Natl. Acad. Sci. USA 90, 8429–8433.
[25] Lütticken, C., Wegenka, U.M., Yuan, J., Buschmann, J., Schindler, C., Ziemiecki, A., Harpur, A.G., Wilks, A.F., Yasukawa, K., Taga, T., Kishimoto, T., Barbieri, G., Pellegrini, S., Sendtner, M., Heinrich, P.C. and Horn, F. (1994) Science 263, 89–92.
[26] Stahl, N., Boulton, T.G., Farruggella, T., Ip, N.Y., Davis, S., Witthuhn, B.A., Quelle, F.W., Silvennoinen, O., Barbieri, G., Pellegrini, S., Ihle, J.N. and Yancopoulos, G.D. (1994) Science 263, 92–95.
[27] Nicholson, S.E., Oates, A.C., Harpur, A.G., Ziemiecki, A., Wilks, A.F. and Layton, J.E. (1994) Proc. Natl. Acad. Sci. USA 91, 2985–2988.
[28] Tanaka, N., Asao, H., Ohbo, K., Ishii, N., Takeshita, T., Nakamura, M., Sasaki, H. and Sugamura, K. (1994) Proc. Natl. Acad. Sci. USA, in press.
[29] Takahashi, T. and Shirasawa, T. (1994) FEBS Lett. 342, 124–128.
[30] Kawamura, M., McVicar, D.W., Johnston, J.A., Blake, T.B., Chen, Y.-Q., Lal, B.K., Lloyd, A.R., Kelvin, D.J., Staples, J.E., Ortaldo, J.R. and O'Shea, J.J. (1994) Proc. Natl. Acad. Sci. USA 91, 6374–6378.
[31] Asao, H., Takeshita, T., Ishii, N., Kumaki, S., Nakamura, M. and Sugamura, K. (1993) Proc. Natl. Acad. Sci. USA 90, 4127–4131.
[32] Maeda, M., Shimizu, A., Ikuta, K., Okamoto, H., Kashihara, M., Uchiyama, T., Honjo, T. and Yodoi, J. (1985) J. Exp. Med. 162, 2169–2174.
[33] Arima, N., Kamio, M., Imada, K., Hori, T., Hattori, T., Tsudo, M., Okuma, M. and Uchiyama, T. (1992) J. Exp. Med. 176, 1265–1272.
[34] Suzuki, J., Takeshita, T., Ohbo, K., Asao, H., Tada, K. and Sugamura, K. (1989) Int. Immunol. 1, 373–377.
[35] Ishii, N., Takeshita, T., Kumura, Y., Tada, K., Kondo, M., Nakamura, M. and Sugamura, K. (1994) Int. Immunol., in press.
[36] Yaegashi, N., Tada, K., Shiraishi, H., Ishii, T., Nagata, K. and Sugamara, K. (1989) Microbiol. Immunol. 33, 561–567.
[37] Shibuya, H., Yoneyama, M., Ninomiya-Tsuji, J., Matsumoto, K. and Taniguchi, T. (1992) Cell 70, 57–67.
[38] Satoh, T., Minami, Y., Kono, T., Yamada, K., Kawahara, A., Taniguchi, T. and Kaziro, Y. (1992) J. Biol. Chem. 267, 25423–25427.
[39] Turner, B., Rapp, U., App, H., Greene, M., Dobashi, K. and Reed, J. (1991) Proc. Natl. Acad. Sci. USA 88, 1227–1231.
[40] Zmuidzinas, A., Mamon, H., Roberts, T.M. and Smith, K.A. (1991) Mol. Cell. Biol. 11, 2794–2803.
[41] Noguchi, M., Yi, H., Rosenblatt, H.M., Filipovich, A.H., Adelstein, S., Modi, W.S., McBride, O.W. and Leonard, W.J. (1993) Cell 73, 147–157.
[42] Schorle, H., Holtschke, T., Hünig, T., Schimpl, A. and Horak, I. (1991) Nature 352, 621–624.
[43] Kondo, M., Takeshita, T., Ishii, N., Nakamura, M., Watanabe, S., Arai, K. and Sugamura, K. (1993) Science 262, 1874–1877.
[44] Kondo, M., Takeshita, T., Higuchi, M., Nakamura, M., Sudo, T., Nishikawa, S. and Sugamura, K. (1994) Science 263, 1453–1454.
[45] Russell, S.M., Keegan, A.D., Harada, N., Nakamura, Y., Noguchi, M., Leland, P., Friedmann, M.C., Miyajima, A., Puri, R.K., Paul, W.E. and Leonard, W.J. (1993) Science 262, 1880–1883.
[46] Noguchi, M., Nakamura, Y., Russell, S.M., Ziegler, S.F., Tsang, M., Cao, X. and Leonard, W.J. (1993b) Science 262, 1877–1880.