# Constitutive Activation of Different Jak Tyrosine Kinases in Human T Cell Leukemia Virus Type 1 (HTLV-1) Tax Protein or Virus-transformed Cells

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## Abstract

**HTLV-1 infection causes an adult T cell leukemia in humans. The viral encoded protein tax, is thought to play an important role in oncogenesis. Our previous data obtained from a tax transgenic mouse model revealed that tax transforms mouse fibroblasts but not thymocytes, despite comparable levels of tax expression in both tissues. Constitutive tyrosine phosphorylation of a 130-kD protein(s) was observed in the tax transformed fibroblast B line and in HTLV-1 transformed human lymphoid lines, but not in thymocytes from Thy-tax transgenic mice. Phosphotyrosine immunoprecipitation followed by Western blot analysis with a set of Jak kinase specific antibodies, identified p130 as Jak2 in the tax transformed mouse fibroblastic cell line and Jak3 in HTLV-1 transformed human T cell lines. Phosphorylation of Jak2 in tax transformed cells resulted from high expression of IL-6. Tyrosine phosphorylation of this protein could also be induced in Balb/c3T3 cells using a supernatant from the B line, which was associated with induction of cell proliferation. Both phosphorylation and proliferation were inhibited by IL-6 neutralizing antibodies. Constitutive phosphorylation of Jak kinases may facilitate tumor growth in both HTLV-1 infected human T cells and the transgenic mouse model. (*J. Clin. Invest.* 1995. 96:1548–1555.) Key words: Jak kinase, HTLV-1 • IL-6 • proliferation • NF-κB**

## Introduction

Human T cell leukemia virus type 1 (HTLV-1) is the etiologic agent of adult T cell leukemia (ATL) (1, 2) and an HTLV-1 associated neurological disorder, called tropical spastic paraparesis or HTLV-1–associated myelopathy (TSP/HAM) (3-4). Mechanisms by which HTLV-1 immortalizes T lymphocytes are not well understood. The virus encoded transcriptional activator protein tax, is considered a crucial factor in tumorigenesis. Tax activates both CREB/ATF and NF-κB transcriptional pathways (5). The CREB/ATF pathway is particularly crucial for regulation of the viral promoter. Activation of NF-κB leads to disregulation of numerous cytokine genes including IL-2/IL-2Rα, IL-3, IL-4, GM-CSF, TNF-α, TGF-β, and IL-6 (6–9). Pervasive up-regulation of these cytokines may favor early growth of infected and neighboring cells and eventually encourage transformation of infected cells.

As demonstrated for HTLV-1–transformed human T cells, tax expressing murine fibroblasts obtained from transgenic mice show disregulation of a similar set of cytokine genes as a consequence of NF-κB activation (10, 11). This leads to fibroblastic tumors in tax transgenic mouse models. Growth of both tax transformed fibroblasts and HTLV-1–transformed human lymphocytes can be blocked by inhibition of NF-κB, but not tax expression (11), suggesting that constitutive activation of NF-κB is crucial for maintenance of the transformed cell growth.

Many NF-κB–regulated soluble growth factors and cytokines have been implicated in autocrine and paracrine growth promoting loops. IL-6, GM-CSF, and IL-2 induce either dimerization of the "β" signal transducing receptor component gp130 with itself (IL-6 and GM-CSF) (12–14) or dimerization of IL-2Rβ-$\gamma_c$ (IL-2, IL-4, IL-7 and IL-9) (15–19). Dimerization of receptor components, in turn activate members of the JAK/TyK family of receptor-associated tyrosine kinases. These consist of Jak1, Jak2, Jak3, and Tyk2 all with sizes of ~ 130 kD (12–19).

In the present study, we show that a 130-kD cellular protein is constitutively tyrosine phosphorylated in both Tax transformed mouse fibroblasts and HTLV-1 transformed human T cells. This protein was identified as Jak2 in tax transformed fibroblasts and Jak3 in HTLV-1 transformed human T cells. Constitutive activation of Jak kinases was a consequence of tax mediated disregulation of IL-6 in the fibroblasts.

## Methods

*Cells.* Cell lines used included Balb/c3T3; the HTLV-1 tax transformed B line (11), cloned from fibrosarcomas occurring in HTLV-1-LTR Tax transgenic mice (10), HTLV-1 transformed human lymphocyte lines: MT2, MT4 (obtained from the American Type Culture Collection, ATCC, Rockville, MD), HUT102, C91-pl, C91-, and C81-66 (provided by Dr. K. McGuire, San Diego State University), and a mature human T cell lymphoma cell line (Jurkat) obtained from ATCC. Mouse thymocytes were isolated from one and a half month old Thy-tax transgenic (20) or normal C57/BL6 mouse thymuses using a protocol previously described (21).

*Antibodies.* Antibodies used in this study were rabbit anti–NF-κB p50 and p65 (from I. Verma, Salk Institute, La Jolla, CA), rabbit anti-Jak1 or Jak2 (UBI NY), rabbit anti-Jak3 (from O'Shea, NCI, FCRF Frederick, MD), mouse monoclonal anti-phosphotyrosine (Transduction Labs), mouse monoclonal anti–IL-6 (2 antibodies obtained from PharMingen, San Diego, CA) and mouse anti–TNF-α (PharMingen).

*ELISA.* A mouse IL-6 ELISA kit (PharMingen) was used to measure secretion of IL-6 in B line cell culture supernatants collected at different time intervals.

*Western blot analysis and immunoprecipitation.* Western blotting

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1. *Abbreviation used in this paper:* HTLV-1, human T cell tropical virus type 1.

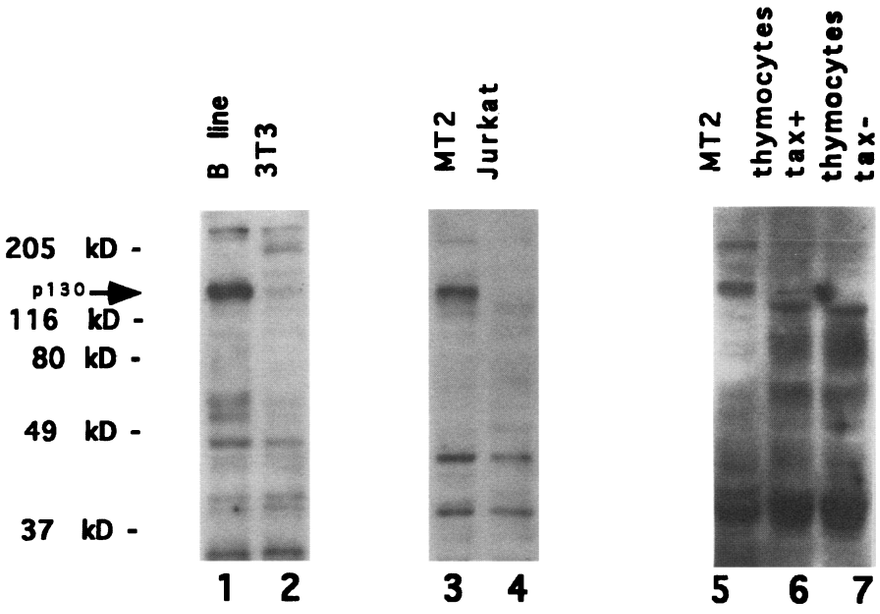


*Figure 1.* A 130-kD cytoplasmic protein is constitutively tyrosine phosphorylated. Western blot analysis of cellular lysates prepared from B line cells (lane *1*), Balb/c3T3 cells (lane *2*), MT2 cells (lane *3*), Jurkat cells (lane *4*), and thymocytes of a Thy-tax transgenic mice (lane *6*) or normal C57/BL mice (lane *7*). 15 $\mu$g of lysate was used for each lane. The blots were probed with anti-phosphotyrosine antibody. The tyrosine phosphorylated p130 is indicated.

was used to detect NF-$\kappa$B p50 and p65, tyrosine phosphorylated proteins and Jak kinases. The tyrosine phosphatase inhibitor sodium orthovanidate (1 mM), was added to the lysis buffer during preparation of the cytosolic extracts. In sequential Immunoprecipitate/Western analyses, detection of tyrosine phosphorylation was performed by immunoprecipitation first, using the phosphotyrosine specific antibody. Briefly, 50 $\mu$g of cytosolic protein was incubated with 1 $\mu$l of mouse anti-phosphotyrosine antibody in RIPA buffer at room temperature for 1 h. The antibodies were captured by incubation with protein G agarose beads (Calbiochem Corp., La Jolla, CA) for another hour at room temperature, followed by washing four times with TBS (0.01 M Tris pH 7.5, 150 mM NaCl, 0.05% Tween 20). Precipitated materials were eluted by boiling in SDS sample buffer and subjected to Western blot analysis using Jak specific antibodies.

*Electrophoretic mobility shift assay (EMSA).* EMSA was performed as previously described (22). Total reaction volume was 25 $\mu$l which included 5 $\mu$g of nuclear extract, 1 $\mu$g of poly (dl-dC), 0.2 ng of $^{32}$P end labeled NF-$\kappa$B probe (AGCTTCAACAGAGGGGACTTTCCGAGAGGCTCGAG) ($10^8$ cpm/$\mu$g DNA). The binding buffer was 10 mM Tris (pH 7.5), 75 mM NaCl, 1 mM EDTA, 7% glycerol, 1 mM DTT. After 20 min of incubation at room temperature, the reactions were loaded on a 5% acrylamide gel and run in 0.5 × Tris-borate-EDTA (TBE) buffer at 4°C. For antibody mediated super shifts, specific NF-$\kappa$B p50 or p65 antibody (2 $\mu$g) was first incubated with nuclear extracts (5 $\mu$g) at room temperature for 20 min. followed by EMSA analysis as above.

*Northern blot analysis.* Poly $(A)^+$ mRNA was isolated from B line cells or Balb/c 3T3 cells using the FastTrack method (Invitrogen, La Jolla, CA). Electrophoresis was followed by transfer onto nylon membranes. Hybridization detection was with a PCR-generated IL-6R probe. Primers were 5′ CCCTGCCCACATTCCTGGT 3′; 5′ TACGGTATTGTCAGACCC 3′. The same blot was re-hybridized with actin probe to control for mRNA loading.

*[$^3$H]Thymidine incorporation assay.* (21) Both B line cells and Balb/c 3T3 cells were plated in 24-well plates for 48 h in DME (GIBCO/BRL, Gaithersburg, MD) plus 10% fetal calf serum DME (GIBCO/BRL) until 80% confluent. Cells were subjected to serum starvation for 24 h by replacing the media with serum free DME. To measure cell proliferation, [$^3$H]thymidine (1 mc$\mu$i/ml) was added to serum starved Balb/c3T3 cells, which were used to monitor cell proliferative response. Undiluted serum free B line cell supernatants or mouse recombinant IL-6 (Genzyme Corp., Boston, MA; 4 ng/ml) were used as sources of growth factors. Inhibition by IL-6 antibody was performed at 10 $\mu$g/ml. Two independent IL-6 neutralizing antibodies yielded similar results. Cells were harvested after 24 h and all assays were performed in triplicate. Experiments were repeated twice. [$^3$H]thymidine incorporation was measured by liquid scintillation counting.

## Results

*A 130-kD protein is constitutively phosphorylated in Tax-transformed mouse fibroblasts and in HTLV-1 transformed human T cell lines.* Previous studies have demonstrated that tax or HTLV-1 expression in some cell lines leads to overexpression of many growth factors and cytokines (6–9). Considerable evidence has also demonstrated that induction of protein tyrosine phosphorylation is associated with growth factor or cytokine-mediated cell proliferation. To evaluate the role of protein tyrosine phosphorylation in HTLV-1 virus or tax transformation, we performed Western blot analyses on several different cell lines, using a phosphotyrosine-specific antibody. Interestingly, a dominant phosphorylated protein with a size of 130 kD was consistently detected in the tax transformed mouse fibroblastic cell lines and in HTLV-1 transformed human T lymphocytes (Fig. 1, lanes *1, 3,* and *5*). This protein was also shown to autophosphorylate itself in situ (data not shown). The protein was not phosphorylated in Balb/c 3T3 (Fig. 1, lane *2*), Jurkat (lane *4*) or normal mouse thymocyte controls (lane *7*). In contrast, some tax expressing but untransformed cells do not overproduce cytokines. In previous studies of a Thy-tax model, we have demonstrated that intra-thymic tax is capable of activating the HTLV-1 LTR promoter (20), presumably through a CREB/ATF pathway (20, 23). However, these tax expressing thymocytes failed to express increased levels of IL-2R, IL-2, IL-6, GM-CSF, or PDGF (20). These Thy-tax thymocytes also failed to show phosphorylation of p130 (lane *6*). Analysis of multiple individual mice gave the same result (data not shown). This suggested that p130 phosphorylation, and cellular transformation was most closely linked to the ability of tax to induce cytokines.

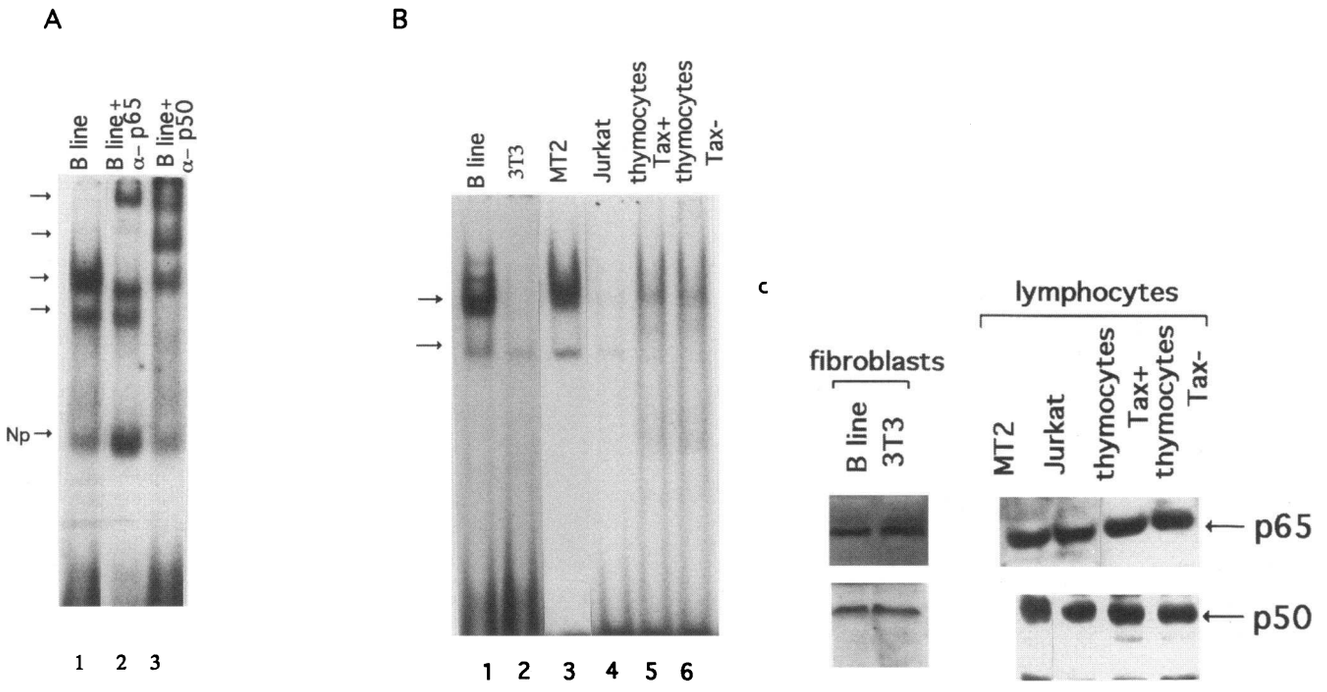


*Figure 2.* Activation of NF-κB in different cells. (*A*) specific nuclear NF-κB binding in tax transformed fibroblasts (*B line*). An Ig-κB ODN containing the consensus sequence for NF-κB was used as an EMSA probe. Two binding complexes were seen in B line nuclear extracts (lane *1*), which can be super shifted by antibodies to p65 or p50 (lanes *2* and *3*). A nonspecific binding complex (*Np*) is indicated by an arrow. (*B*) EMSA in different cells. Nuclear extracts from B line (lane *1*), Balb/c3T3 (lane *2*), HTLV-1–transformed human T lymphocytes (*MT2,* lane *3*), Jurkat cells (human T cell leukemia cell line, lane *4*) and thymocytes from a tax positive Thy-tax transgenic mouse (lane *5*) and a normal C57/BL mouse (lane *6*). Equal amounts of nuclear extract (5.0 μg) were applied to each lane. The sequence of NF-κB probe is described in the methods section. (*C*) Western blot analysis with antibodies to NF-κB p50 and p65. Tested cell lines and p50 or p65 protein are indicated.

*Phosphorylation of p130 associated with nuclear activation of NF-κB.* In addition to activating CREB/ATF family members, tax has been shown to be a potent activator of the NF-κB pathway. Indeed, the cytokines best associated with tax or HTLV-1 transformation are NF-κB dependent. To examine correlation between p130 phosphorylation and nuclear activation of NF-κB, we performed EMSA analyses. We used a $^{32}$P-labeled probe which contains the NF-κB consensus sequence of the Ig-κB promoter and has been demonstrated to bind the p50/p65 heterodimer efficiently (11). As shown in Fig. 2, tax-transformed mouse fibroblastic tumor cells and HTLV-1 transformed human T cells revealed constitutive nuclear binding to this probe. This binding could be super-shifted by antibodies to both p65 and p50 (Fig. 2 *A,* lanes *2* and *3*), and specifically competed by wildtype NF-κB ODN but not mutant NF-κB ODN (data not shown). In contrast, neither Jurkat cells, thymocytes from normal or Thy-tax transgenic mice nor Balb/c 3T3 cells showed high levels of NF-κB binding activity (Fig. *2 B,* lanes *2, 4, 5,* and *6*). Overall expression levels of NF-κB p50 and p65 were the same in all cells, as judged by Western blot analyses (Fig. *2 C*). This suggests that activation of NF-κB by tax may be necessary for tyrosine phosphorylation of p130. Tissue specific inability of tax to activate the NF-κB pathway in thymocytes was associated with failure to phosphorylate p130 and transform these cells.

*IL-6 secreted by Tax-transformed transgenic mouse fibroblasts, induces phosphorylation of p130 and cellular proliferation.* As shown above, a signal transduction pathway activated by NF-κB may mediate phosphorylation of p130. A good candidate for a mediator in tax transformed transgenic fibroblasts was IL-6, since we have previously shown its expression to be highly stimulated in tax-transformed fibroblasts (11, 24). In addition, its expression is highly NF-κB dependent in tax transformed cells, since inhibition of the p65 component of NF-κB by antisense oligodeoxynucleotides resulted in profound decrease of IL-6 expression in this cell line (11). Using a murine specific ELISA, we determined the accumulation rate of IL-6 in culture supernatants to be 0.2 ng/ml per hour (Fig. *3 A*). IL-6 expression was not detected in supernatants from control Balb/c3T3 cells (Fig. *3 A*). Both tax transformed fibroblasts and Balb/c3T3 cells expressed similar levels of the p80 IL-6 receptor (Fig. *3 B*). Therefore, both should be equally capable of responding to IL-6.

To establish whether p130 phosphorylation could be caused by secreted IL-6, supernatants from the tax transformed fibroblast (B line) cells were added to Balb/c3T3 cell cultures. Within 1 h, p130 was the most prominent protein observed to be tyrosine phosphorylated in Balb/c 3T3 cells (Fig. 4). To evaluate the role of IL-6 in inducing phosphorylation of p130, the ability of IL-6 neutralizing antibodies to inhibit p130 phosphorylation was evaluated. A similar class and concentration of NF-κB p50 or TNF-α antibodies were used as controls. Both the IL-6 and TNF-α antibodies are known to be neutralizing and are extremely clean when analyzed by Western blot or ELISA (data not shown). As shown in Fig. 4, only IL-6 antibody was able to specifically block tyrosine phosphorylation of p130 in Balb/c3T3 cells (lanes *7* and *8*). This suggested that tyrosine phosphorylation of p130 was largely mediated by solu-

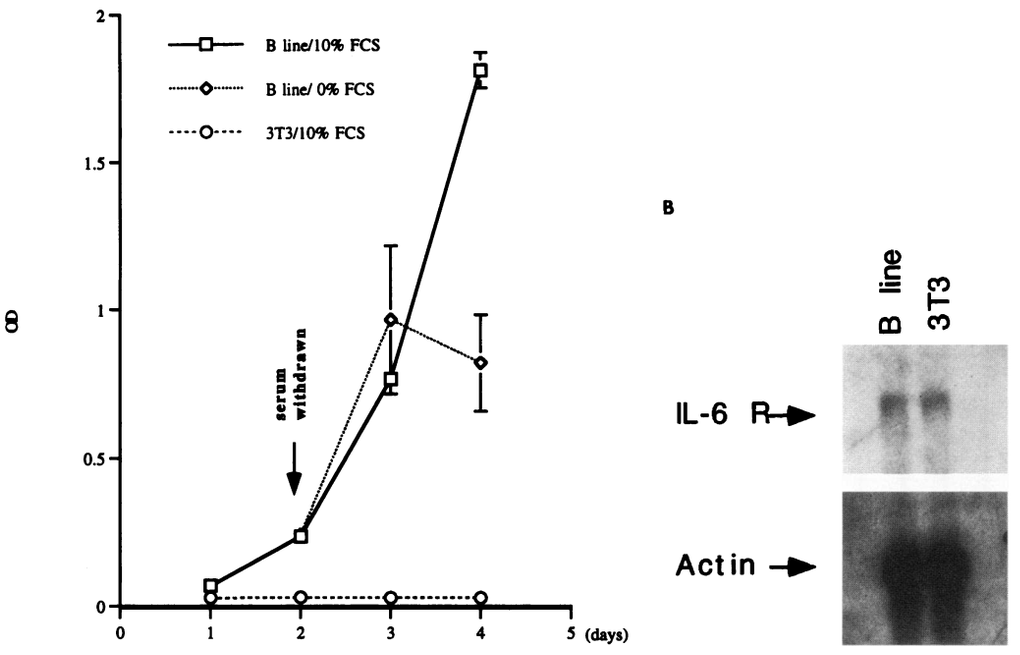


*Figure 3.* IL-6 and IL-6 receptor expression in murine fibroblasts. (*A*) Kinetics of IL-6 secretion in B line cells, cultured with or without serum. Balb/c3T3 cultures were used as controls for ELISAs. Cells were grown in the presence of 10% FCS until day 2 when cells were 60–80% confluent. The concentration of secreted IL-6 grown in 10% serum or serum free conditions was calculated using an IL-6 standard curve (not shown here). (*B*) Northern blot analysis of the IL-6 receptor. 2.0 μg of poly(A)$^+$ mRNA obtained from B line cells or Balb/c 3T3 cells were hybridized with a 220-bp fragment of mouse IL-6R cDNA (*top panel*) and an actin cDNA control (*bottom panel*).

ble IL-6 secreted by tax transformed fibroblasts. Indeed, phosphorylation of p130 in Balb/c3T3 could be equally induced by either B line supernatant or recombinant IL6 but not by serum or PMA (data not shown).

The relationship between IL-6 induced phosphorylation of p130 and cell proliferation was then studied. [$^3$H]thymidine incorporation experiments were performed. The above described ELISA results, demonstrated secretion of IL-6 by B cells even when grown in the absence of serum up till day 3 of culture (Fig. *3 A*). This allowed stimulation to be measured in the absence of exogenous serum. Balb/c3T3 cells were allowed to reach 80% confluence prior to serum starvation for 24 h. Under these conditions, cell growth was largely arrested (Fig. *5*, lane *1*). When either the serum free supernatant from B line cell cultures or recombinant IL6 were added, [$^3$H] thymidine incorporation rate in Balb/c3T3 cells was increased dramatically (lanes *3* and *5*). This increase was specifically inhibited by IL-6 neutralizing antibody (lanes *4* and *6*). These results demonstrate that tax induced IL-6 may play a crucial role in paracrine stimulation of untransformed fibroblasts, and that this may be mediated through p130 phosphorylation.

*Phosphorylated p130 identified as Jak2 in the Tax-transformed transgenic mouse fibroblasts and as Jak3 in HTLV-1–transformed human T cells.* Recently, Jak kinases have been shown to be involved in the cytokine signaling pathway for IL-6 (12–14) IL-2, IL-4, IL-7, and IL-9 (15–19). The similarity in size between Jak family members and our p130 led us to investigate whether our tyrosine phosphorylated protein belongs to this family. Sequential phosphortyrosine immunoprecipitation followed by Western blot analysis using Jak1, Jak2, and Jak3 antibodies were performed on cellular extracts. Tyrosine phosphorylation of Jak2 was observed in tax transformed fibroblasts (B line), but not in Balb/c3T3 cells (fig. 6A, left panel). Both cells expressed equal amounts of total Jak2 (fig. 6A, right panel). B line cells showed a high level of Jak2 protein which comigrates with phosphorylated p130 (Fig. *6 B* lanes *2* and *4*), and undetectable levels of Jak3 and Jak1 (Fig. *6 B*, lanes *1* and *3*).

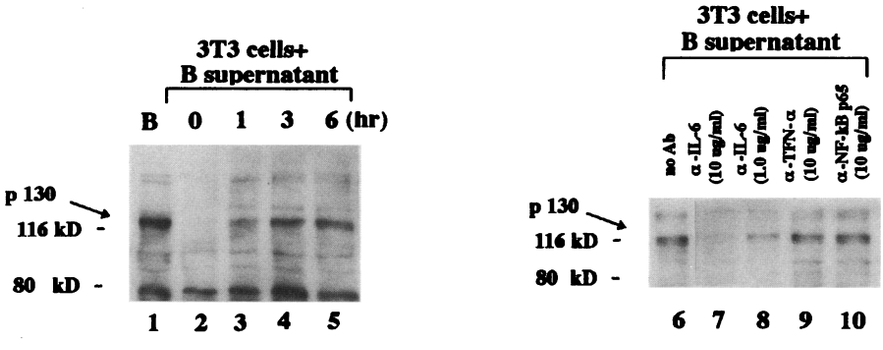


*Figure 4.* Induction of p130 phosphorylation in Balb/c3T3 cells. Balb/c3T3 treated with B supernatants from 0 to 6 h (lanes *2/5*). Western blot analysis using phosphotyrosine antibody. B line cell lysates were used as positive controls (lane *1*). Inhibition of p130 phosphorylation induction by neutralizing antibodies (lanes *6–10*). Equal amount of lysates (15 μg) were used in each lane. The location of tyrosine phosphorylated p130 is indicated by arrows.

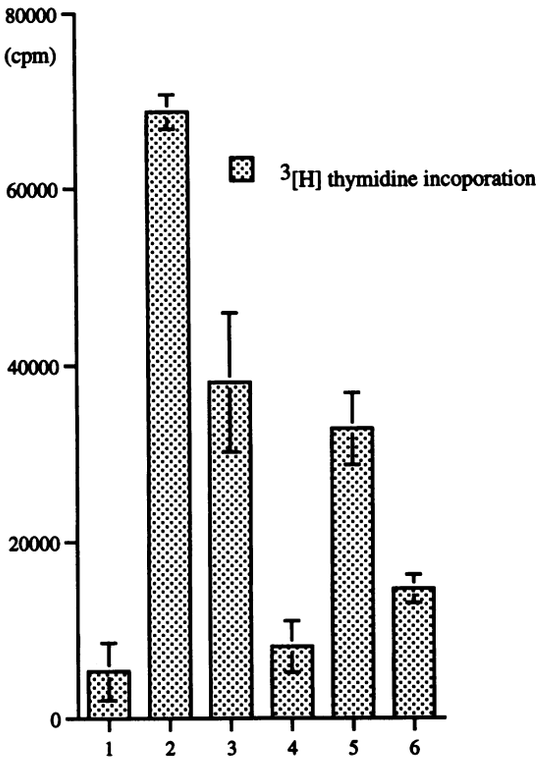


*Figure 5.* Proliferation of Balb/c3T3 cells is stimulated by B line cell supernatants or mouse recombinant IL-6. [$^3$H]thymidine incorporation was measured in Balb/c3T3 cells grown in 10% FCS media (lane *2*), serum free media (lane *1*), mouse recombinant IL-6 (lane *3*), mouse recombinant IL-6 with neutralizing IL-6 antibody (line *4*), serum free B line cell supernatants without IL-6 antibody (lane *5*), or with IL-6 antibody (lane *6*). Cells were harvested after overnight incubation with [$^3$H]thymidine. The data shown are the means of three determination±SD in a representative experiment.

Since the HTLV-1 transformed human lymphocyte line MT2, shows phosphorylation of a similar p130 (Fig. 1), we performed identical immunoprecipitate/Western analyses of lymphocytes. These results demonstrated highly phosphorylated Jak3 in HTLV-1 transformed MT2 and MT4, but only weak phosphorylation in Jurkat cells (Fig. 7 *A*, lanes *1–3*). Jak2 was also weakly phosphorylated in MT2 and MT4, but not in Jurkat (Fig. 7 *A*, lanes *4–6*), whereas Jak1 expression and phosphorylation were undetectable (Fig. 7 *A*, lanes *7–9* and Fig. 7 *B*, lane *1*). In MT2 cells, total protein expression of Jak3 was also increased (Fig. 7 *B*). The weak phosphorylation of the nondominant Jak 2 in MT2 and MT4 cells, may result from previously described aberrant expression of IL-6 in HTLV-1–infected T cells (9), or may reflect true interaction between Jak3 and Jak2 kinases in this system. Constitutive phosphorylation of Jak3 was also seen in four other prototypic HTLV-1 transformed human cell lines, Hut102, C81-66, C91, and C91-pl (Fig. 7 *C*), and therefore appears to be a common event in HTLV-1 transformation. In contrast to HTLV-1 transformed T-cells, immature murine thymocytes do not express Jak3 protein (Fig. 7 *D*).

## Discussion

We have shown that transformation by either the tax gene or HTLV-1 virus is associated with constitutive tyrosine phosphorylation of Jak kinase family members. The dominant species is Jak2 in tax transformed fibroblasts and Jak3 in HTLV-1–transformed human lymphocytes. Phosphorylation of the IL-6 gp130 receptor may also contribute to the signal seen on crude phosphotyrosine blots. These results provide the first evidence that specific Jak kinase activation may be involved in HTLV-1 or tax transformation.

Jak/Tyk family members have been previously shown to propagate growth factor and cytokine mediated effects on mitogenesis and differentiation (25, 26). Particular cytokines appear to induce distinct patterns of Jak/Tyk phosphorylation in different cell lines. For example, tyrosine phosphorylation of Jak1 and Jak2 but not Tyk2 was always observed after stimulation with CNTF, IL-6, LIF, and OSM (12). On the other hand, stimulation of INF-$\alpha$ usually induces phosphorylation of Tyk2 and Jak1 but not Jak2 (27, 28). Jak3 activation appears restricted to mature lymphocytes and is linked to activation by IL-2, IL-4, IL-7, and IL-9 (16, 29). In tax-transformed mouse fibroblasts, phosphorylation of Jak2 but not other family members appeared dominant, whereas both up modulation and phosphorylation of Jak3 was dominant in HTLV-1 transformed human T cells.

In tax transformed mouse fibroblasts, activation of Jak2 occurs in response to IL-6. IL-6 expression has been demonstrated to be highly up-modulated in these cells, at least partially as a consequence of NF-$\kappa$B activation (11, 24, 30) by tax. Results from ELISA reveal high levels of IL-6 secreted into the media even when these cells are serum starved. Furthermore, IL-6 receptor mRNA was also detected. Thus, the interaction of IL-

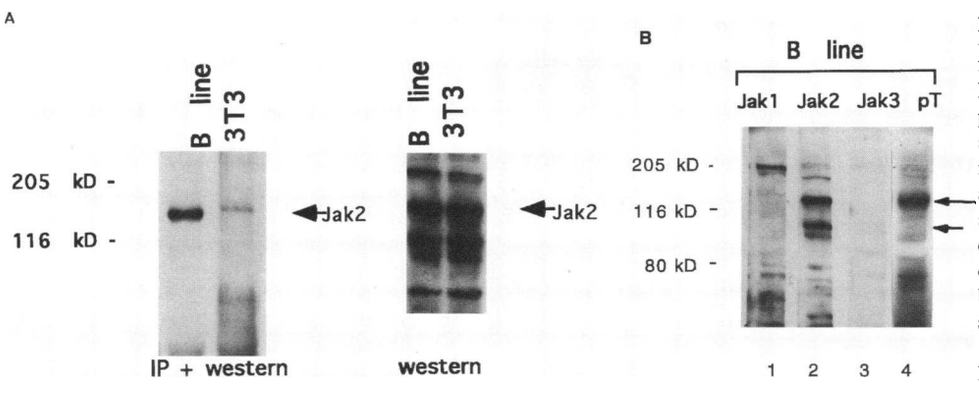


*Figure 6.* Identification of phosphorylated Jak2 in Tax transformed mouse fibroblasts. (*A*) 50 $\mu$g of B line or Balb/c3T3 cell lysates were immunoprecipitated with a phosphotyrosine specific antibody followed by immunoblot detection with a Jak2 specific antibody (*left panel*). The right panel shows for comparison, total Jak2 levels in 15 $\mu$g of B line or Balb/c3T3 cell lysates. Jak2 protein is indicated by arrows. (*B*) Western blot comparison of Jak proteins in the B line cells. 15 $\mu$g of cellular lysate were used per lane. Antibodies are indicated.

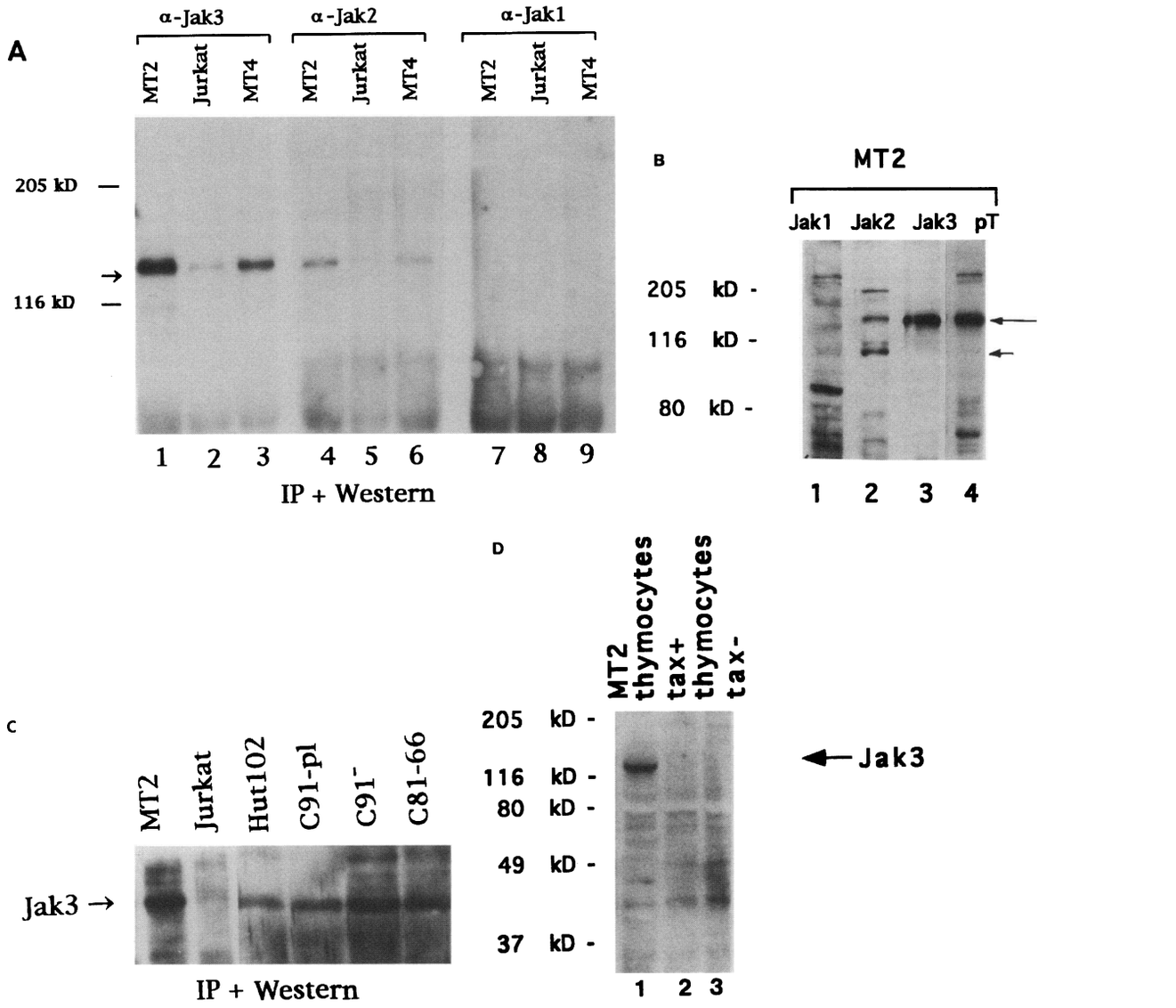


*Figure 7.* Identification of Jak species in HTLV-1 transformed human lymphocytes and murine tax expressing thymocytes. (*A*) Phosphorylation of Jak1-3 in MT2, MT4 and Jurkat cells. 50 μg of cell lysates were immunoprecipitated with phosphotyrosine antibody followed by Western blotting analysis with Jak3 (lanes *1–3*), Jak2 (lanes *4–6*) and Jak1 (lanes *7–9*) antibodies. Phosphorylated Jak kinases were indicated by an arrow. (B) Western blot comparisons of Jak1-3 total protein levels in MT2 (lanes *1–4*) Jak1 (lane *1*), Jak2 (lane *2*), Jak3 (lane *3*), or phosphotyrosine (lane *4*) antibodies. 15 μg of MT2 or B line cell lysates were used in each lane. The location of corresponding Jak kinases is indicated by a long arrow. The shorter arrow indicates degraded Jak2. (*C* Phosphorylation of Jak3 in four more HTLV-1 transformed human T-cells (Hut102, lane *3*; C91-pl, lane *4*; C91-, lane *5*; and C81-66, lane *6*). This time MT2 (lane *1*) and Jurkat (lane *2*) cell lysates were used as positive and negative controls. The phosphorylated Jak3 is indicated by an arrow. (*D*) Expression of Jak3 in thymocytes from Thy-tax transgenic mice (lane *2*) normal mice (lane *3*) or MT2 cell lysates (lane *1*). Direct Western blot analysis using Jak3 specific antibodies.

6 with its cognate receptor in tax transformed mouse fibroblasts may further stimulate cells by the Jak2 signaling pathway. In vitro stimulation by supernatants from B line cell cultures induced specific phosphorylation of Jak2 in Balb/c 3T3 cells, and simultaneous proliferation. Both phosphorylation and proliferation were specifically inhibited by IL-6 neutralizing antibody suggesting a direct correlation between activation of the autocrine loop and cell proliferation. Constitutive activation of Jak2 by IL-6/IL-6R may not only result in autocrine cell proliferation but may also cause paracrine effects on other cells. For example, hyperplasia of bone stroma, and multiple hematopoetic bone marrow precursors, as well as muscle wasting are commonly seen in transgenic mice bearing IL-6 secreting fibrosarcomas.

IL-6, IL-2, and IL-2Rα are well known to be up-modulated by NF-κB in tax or HTLV-1 transformed cells (5, 6, 11). Indeed, tax may release NF-κB from an inactive complex by physically interacting with I-κB. In contrast, IL6 does not induce NF-κB in normal fibroblasts or in tax transformed cells (data not shown), nor has it been reported by others to our knowledge. Thus, NF-κB is not capable of activating itself through this loop. In our studies, the role of tax appears to be activation of NF-κB which in turn induces IL6. High level

IL-6 expression then leads to Jak2 phosphorylation. Factors downstream of Jak kinases such as Stat and MAP kinase family members remain to be elucidated. In addition, other IL-6 independent mechanisms of tax may play a role in transformation. For example, Jak2 kinase may also be partially activated by other overexpressed cytokines. Their resulting signaling cascades might cause further disregulation of cellular genes (37, 38) involved in tumorigenesis.

Tyrosine phosphorylation of Jak3 has been associated with T cell activation by IL-2, IL-4, IL-7, and IL-9 through the IL-2R (15–19). The IL-2 receptor consist of an $\alpha$-chain, $\beta$-chain, and $\gamma$-chain (32). Expression of the $\alpha$-chain is highly upregulated by tax/rex gene products in HTLV-1 transformed human T lymphocytes (6, 33). However, upregulation of the $\gamma_c$ chain has also been linked to HTLV-1 infection and tax activation (34). Although induction of IL-2 and IL-2R$\alpha$ are important events in activation of T cells (35, 36), it is the $\beta$- and $\gamma_c$-chain that mediate signal transduction (15–19). The membrane-proximal, serine-rich domains of these proteins interact with Jak3 kinase and are required for cytokine mediated mitogenesis (16). In addition to IL-2, IL-6, and IL-2R$\alpha$, $\gamma$, HTLV-1 infected T cells have been reported to express a multitude of other cytokines including IL-3, IL-4, IL-5, TNF-$\beta$, and GM-CSF (8). Among them, IL-4 was recently reported to activate T-cell proliferation by activation of Jak3 through the IL-2$\gamma$ chain (17–19). IL-15 may activate similarly (41, 42). Thus, in HTLV-1–transformed human lymphocytes, constitutive activation of Jak3 is most likely a result of disregulation of one or more of these cytokine genes in addition to the IL-2 receptor subunit genes. Furthermore, in human T cells infected with whole virus, virus encoded protein(s) other than tax may also contribute to activation of Jak3 (39, 40).

Future identification of additional downstream cytokine and other targets of tax in additional leukemic cell lines and fresh patient isolates will be critical for understanding pathogenesis and therapeutic approaches to this unusual leukemia.

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