

Txk, a Nonreceptor Tyrosine Kinase of the Tec Family, Is Expressed in T Helper Type 1 Cells and Regulates Interferon γ Production in Human T Lymphocytes

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Summary

Differentiation of human T cells into T helper (Th)1 and Th2 cells is vital for the development of cell-mediated and humoral immunity, respectively. However, the precise mechanism responsible for the Th1 cell differentiation is not fully clarified. We have studied the expression and function of Txk, a member of the Tec family of nonreceptor tyrosine kinases. We found that Txk expression is restricted to Th1/Th0 cells with IFN- γ producing potential. Txk transfection of Jurkat T cells resulted in a several-fold increase of IFN- γ mRNA expression and protein production; interleukin (IL)-2 and IL-4 production were unaffected. Antisense oligodeoxynucleotide of Txk specifically inhibited IFN- γ production of normal peripheral blood lymphocytes, antigen-specific Th1 clones, and Th0 clones; IL-2 and IL-4 production by the T cells was unaffected. Txk cotransfection led to the enhanced luciferase activity of plasmid (p)IFN- γ promoter/enhancer (pIFN- γ [-538])-luciferase-transfected Jurkat cells upon mitogen activation. Txk transfection did not affect IL-2 and IL-4 promoter activities. Thus, Txk specifically upregulates IFN- γ gene transcription. In fact, Txk translocated from cytoplasm into nuclei upon activation and transfection with a mutant Txk expression plasmid that lacked a nuclear localization signal sequence did not enhance IFN- γ production by the cells, indicating that nuclear localization of Txk is obligatory for the enhanced IFN- γ production. In addition, IL-12 treatment of peripheral blood CD4⁺ T cells enhanced the Txk expression, whereas IL-4 treatment completely inhibited it. These results indicate that Txk expression is intimately associated with development of Th1/Th0 cells and is significantly involved in the IFN- γ production by the cells through Th1 cell-specific positive transcriptional regulation of the IFN- γ gene.

Key words: Txk • gene transcription • T helper 1/T helper 2 cells • human • interferon γ

Thelper type 1 cells are characterized by their secretion of IFN- γ and induce macrophage cytotoxicity, delayed-type hypersensitivity, and enhanced cellular immunity (1–3). It has been suggested that cytokines and their receptors, transcription factors, MHC determinants, Ag peptides, and costimulatory signals are important for Th1 and Th2 cell differentiation (1–9). However, to date, the precise mechanism responsible for the differentiation and development of polarized Th1 responses has not been fully clarified in humans.

The Tec family has emerged recently as a subfamily of nonreceptor tyrosine kinases and consists of Tec, Btk, Itk/Tsk/Emt, Bmx, and Txk/Rlk (10–25). Because many members of this family have been shown to be activated in response to growth and differentiation stimuli in hematopoietic tissues, they are presumed to function in vivo as im-

portant signaling mediators (17); indeed, mutations in Btk cause agammaglobulinemia in humans (18, 25).

Information concerning roles of Txk in human T lymphocyte function is limited. Txk displays highly cell type-specific expression largely restricted to T cells and some mast cell and myeloid cell lines (10, 11, 17). Txk has src homology (SH)2¹ and SH3 domains and a nuclear localization signal sequence but lacks a pleckstrin homology domain (10–12, 17). The NH₂ terminus of Txk in humans and that of Rlk, a mouse homologue of human Txk, possess an unusual cysteine-rich string, suggesting that Txk/Rlk functions in a manner that differs from the other pleckstrin

¹Abbreviations used in this paper: CAT, chloramphenicol acetyl transferase; ODN, oligodeoxynucleotide; p, plasmid; RSV, Rous sarcoma virus; RT, reverse transcription; SH, src homology.

homology domain-containing Tec family kinases (10–12, 17). More recently, Schneider et al. reported that Rlk is capable of phosphorylating CTL-associated antigen (CTLA)-4, suggesting that Rlk may participate in CTLA-4 function (15).

The finding that mouse Rlk is expressed on Th1 but not Th2 cells (10) prompted us to study the role of Txk in Th1 responses in humans. In this study, we found that Txk expression is restricted to Th1/Th0 cells and that Txk is involved in the regulation of Th1 cytokine production by human T lymphocytes.

Materials and Methods

Establishment of Ag-specific T Cell Clones and Cytokine Production. House dust mite-specific, Japanese cedar pollen *Cryptomeria japonica* 1-specific, and purified protein derivatives of tuberculosis (PPD)-specific T cell clones were established by a standard procedure using limiting dilution technique (26). All of the clones expressed TCR- α/β , CD3, CD4, and CD45RO but were negative for CD8. To induce cytokine production, the clones were stimulated with the relevant Ag plus irradiated autologous PBMCs and, in some experiments, irradiated autologous purified monocytes as APCs for 18–24 h. IL-2 (BioSource International), IL-4 (R & D Systems, Inc.), and IFN- γ (BioSource International) levels of culture supernatants were measured using ELISA kits. We assigned Th0, Th1, and Th2 as follows (26): Th1 clones that produce IFN- γ and undetectable IL-4 (<10 pg/ml); Th2 clones that produce IL-4 and undetectable IFN- γ (<10 pg/ml); and Th0 clones that produce both IL-4 and IFN- γ .

Purification of CD3⁺, CD4⁺CD3⁺, and CD8⁺CD3⁺ T Cells. PBMCs were separated into sheep red blood cell (SRBC)-rosetted cells and unrosetted cells. CD3⁺ T cells were purified from SRBC-rosetted cells by magnetic bead depletion (MBD) of CD11a, CD14, CD19, and CD56 cells (27). CD4⁺CD3⁺ and CD8⁺CD3⁺ T cell subsets were similarly purified by the MBD technique. The resulting cell populations were always >97% pure for cells of the relevant phenotype.

Construction of Wild-Type and Mutant Txk Expression Vectors. Human Txk cDNA in λ phage was provided by Dr. G.W. Litman (University of South Florida, St. Petersburg, FL) (11). Full length Txk cDNA was amplified by PCR using a sense primer (CGGAATTCATGATCCTTTTCTCCTATAACA) and an anti-sense primer (TTCTCTAGATCACCAGGTTTCCGCAAT-CTC), and the product was ligated into a mammalian expression vector, pME18S (SR- α promoter; provided by Dr. K. Maruyama, Tokyo Medical and Dental University, Tokyo, Japan) (28). The vector pME18S-Txk carries the full length wild-type human Txk cDNA.

The Txk mutant was created using the QuickChange site-directed mutagenesis kit (Stratagene Inc.). In brief, pME18S-Txk was used as a template. The primers containing the desired mutation were employed for PCR amplification using PfuTurbo DNA polymerase (Stratagene Inc.). The amplification cycle consisted of 1 cycle of denaturation (95°C) for 1 min, followed by 18 cycles of denaturation (95°C) for 30 s, annealing for 1 min (55°C), and polymerization for 10 min (68°C). After PCR cycling, the PCR product was treated with DpnI, which is specific for methylated and hemimethylated DNA, and the synthesized nonmethylated DNA containing the desired mutation was recovered. The resultant mutant vector was used for transformation of *Escherichia coli* DH5 α .

A part of the hypothetical nuclear localization sequence of

Txk, KRKP, was deleted from the wild-type Txk, and the rest of Txk cDNA was kept intact (16, 17, 29). The primers used for constructing the deletion mutant were as follows: KRKP-deletion, 5'-CGGGCCGTGTGCAGCCGTCCTGCCCTCCCC-TCCCACCCTC-3' and 5'-GAGGGTGGGAGGGGAGGC-AGTGACGGCTGCACACGGCCCCG-3'. Fidelity of all the constructs was confirmed by DNA sequencing.

Transfection into Cell Lines and Luciferase Assay. Purified plasmids were transfected into Jurkat and Raji cells by electroporation as described (28). After a 48-h incubation, cells were collected, counted, and stimulated with PHA (1 μ g/ml) plus PMA (10 ng/ml) or PMA (10 ng/ml) plus ionomycin (1 μ g/ml) for 24 h to induce lymphokine production. 5 μ g of plasmid (p)IFN- γ (-538)-luciferase (provided by Dr. C.B. Wilson, University of Washington, Seattle, WA), 5 μ g pRSV (Rous sarcoma virus)-chloramphenicol acetyl transferase (CAT), and 10 μ g of pME18S-Txk (Txk transfection) or pME18S (empty vector; mock transfection) was cotransfected into Jurkat cells.

pIL-2(-568)-luciferase (provided by Dr. C.B. Wilson) with pRSV-CAT and pIL-4(-265)-CAT (provided by Dr. S.N. Georas, Johns Hopkins University, Baltimore, MD) with pGL-3 control vector (SV-40 promoter; Promega Corp.) were similarly transfected. 48 h after transfection, the Jurkat cells were stimulated with PHA plus PMA for 8 h. Thereafter, luciferase assay and CAT-ELISA (Roche Diagnostics) of the cells were carried out (30).

Antibodies. Txk expression of transfected cells and normal lymphocytes was studied by immunoblotting (28), immunocytochemical staining (31), and immunofluorescence analysis using goat anti-Txk Ab (Santa Cruz Biotechnology). Fluorescence-conjugated anti-IFN- γ mAb (Immunotech) was used for intracytoplasmic IFN- γ staining.

Immunofluorescence Staining of Intracytoplasmic Proteins. Immunofluorescence staining of intracytoplasmic proteins was carried out by a modification of the method of Sander et al. (32). In brief, the cells were fixed by using 4% paraformaldehyde and permeabilized by 0.1% saponin (Sigma Chemical Co.) in PBS with 0.01 M Hepes buffer solution. Thereafter, intracytoplasmic antigens were stained with purified first Abs, biotin-conjugated second Abs, and streptavidin-fluorochrome. Thereafter, the cells were analyzed by flow cytometry. Appropriate control Abs were included to define the background immunofluorescence of the cells in this study.

Immunocytochemical Staining. T cells or T cell clones were recovered and cytospin preparations of them were made. The samples were fixed with cold acetone for 15 min and were blocked with 2% skim milk for 30 min. The samples were incubated with first Abs overnight at 4°C. All subsequent procedures were performed using an LSAB kit (DAKO JAPAN).

Reverse Transcription-PCR Analysis. IFN- γ mRNA expression of Jurkat cells was estimated by reverse transcription (RT)-PCR using limiting dilutions of cDNA to accurately estimate the relative amounts of mRNA expression in different samples as previously reported (31). Txk-transfected and mock-transfected Jurkat cells were cultured for 48 h and then stimulated with PHA plus PMA for 8 h. Total RNA was extracted from these cells, and was cDNA synthesized. The sequences of IFN- γ , IL-4, and β -actin primers and PCR conditions were reported previously (33). For amplification of Txk cDNA, the following primers were used: Txk sense, TTGCTGTTTCAGTGCAGAA; Txk antisense, GCA-CCTTCTTTAGACTCT; 475-bp product.

Antisense Oligodeoxynucleotide and Cell Culture. Sense (GGG-CTACCATGAGGTTTC) and antisense (GAAACCTCATGG-

TAGCCC) oligodeoxynucleotides (ODNs) specific for T_{xk} were synthesized as a sulfonated form. Peripheral blood T cells and Ag-specific cloned T cells (10⁶ cells/ml) were incubated in the presence of various concentrations of ODNs for several hours. Thereafter, peripheral blood T cells were stimulated with PHA (1 μg/ml) and Ag-specific cloned T cells with irradiated autologous PBMCs plus the optimal concentration of Ag (26).

Results and Discussion

We first studied T_{xk} expression of various cell types. Jurkat and MOLT-4 cells (T cell lines) but not Raji (a B cell line)- nor EBV-transformed B cells expressed T_{xk}. Both peripheral blood CD4⁺ and CD8⁺ T cells expressed T_{xk}; peripheral blood B cells and monocytes, however, never expressed it. We next asked whether T_{xk} expression is restricted to a certain T cell subpopulation, such as Th1 cells. Various Ag-specific T cell clones that had been cultured for 7 d after the last stimulation with irradiated autologous PBMCs plus the relevant Ags were recovered. RT-PCR analysis revealed that all 20 Th1 cell clones and all 20 Th0 clones tested expressed T_{xk} mRNA, whereas none of the 20 Th2 clones expressed it (Fig. 1 a).

Immunocytochemical staining confirmed that all Th1 and Th0 clones with various Ag specificities from several donors expressed T_{xk}; all of these clones have IFN-γ pro-

ducing potential (Fig. 1, a and b). However, Th2 clones, regardless of Ag specificity or the donor, did not express T_{xk} at all (Fig. 1, a and b). Thus, there is an intimate association between T_{xk} expression and Th1/Th0 clones with IFN-γ producing potential.

We next studied the effects of T_{xk} overexpression on cytokine production by T cells. To this end, Jurkat cells were transfected with pME18S-T_{xk}. We used Jurkat cells in these experiments because they produce relatively low levels of Th1 cytokines upon mitogen activation. Overexpression of T_{xk} by the transfection was confirmed by immunoblotting with anti-T_{xk} Ab (Fig. 2 a). In parallel experiments, Jurkat cells were stimulated with PHA plus PMA. When we used ELISA to detect cytokine secretion, T_{xk} transfection resulted in several-fold more IFN-γ production as compared with the mock transfection (Fig. 2 b). We also confirmed that T_{xk} transfection of Jurkat cells enhances IFN-γ production by using an IFN-γ-specific ELISpot assay (data not shown).

Similarly, intracytoplasmic IFN-γ staining of the Jurkat cells was carried out. As shown in Fig. 2 c, T_{xk} transfection of Jurkat cells led to a several-fold increase of intracytoplasmic IFN-γ-positive cells; in mock-transfected Jurkat cells stimulated with PHA plus PMA, 13% were intracytoplasmic IFN-γ-positive cells; in T_{xk}-transfected Jurkat cells stimulated with PHA plus PMA, 36% were intracytoplasmic-

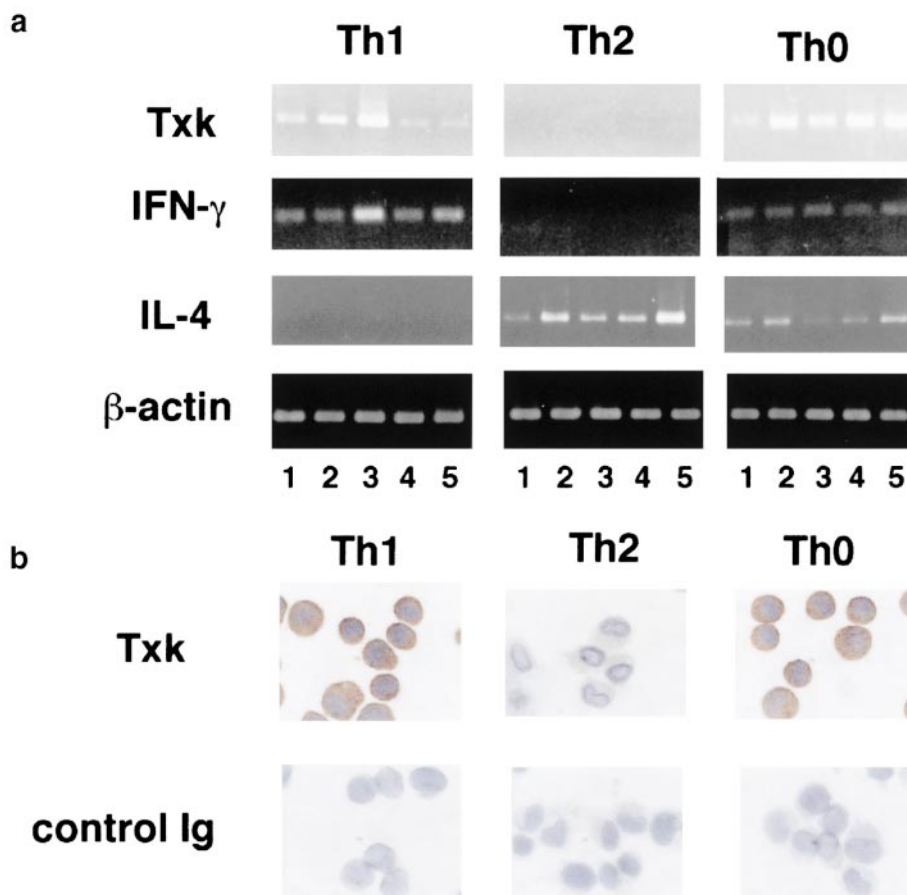


Figure 1. Analysis of T_{xk} expression of human Ag-specific T cell clones. (a) RT-PCR analysis of T_{xk} expression of human Th1, Th2, and Th0 cell clones. Unstimulated T cell clones were used for analysis of T_{xk} mRNA expression. T cell clones were stimulated for 12 h with the relevant Ag plus irradiated monocytes as APCs and analyzed for cytokine mRNA expression. Results of five Th1, five Th0, and five Th2 representative clones of various antigen specificities from several different donors are shown. (b) Representative Th1, Th2, and Th0 cell clones were stained by the immunocytochemical method for T_{xk} protein expression (magnification 250). We studied a total of 20 Th1, 20 Th0, and 20 Th2 clones with various Ag specificities from several donors. All of the Th1 cell clones were strongly positive for T_{xk} expression, whereas none of Th2 clones expressed it. All of the Th0 clones were positive for T_{xk} expression with various staining intensities.

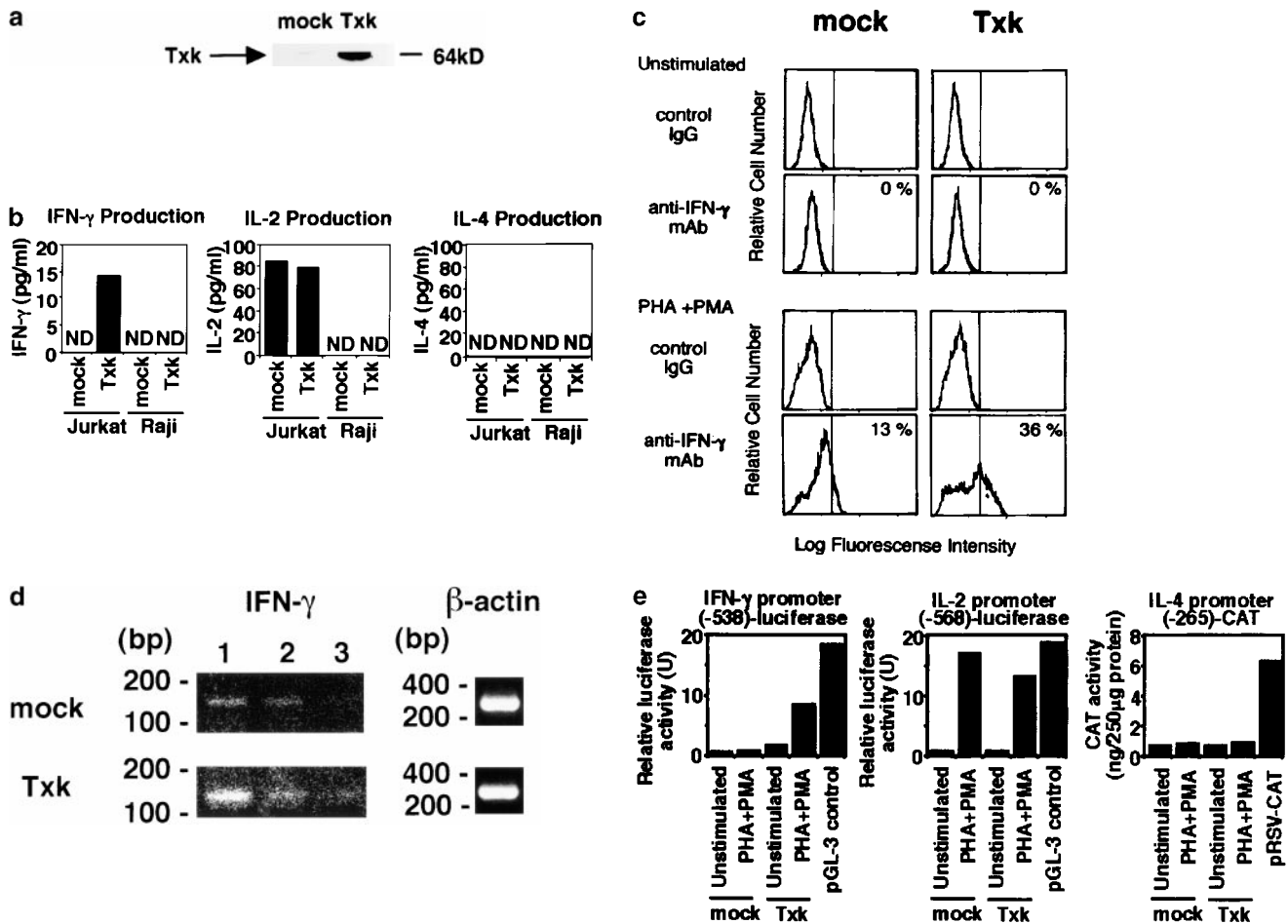


Figure 2. Effects of Txk transfection on IFN- γ production by Jurkat cells. (a) Jurkat cells were transfected with pME18S (mock) and pME18S-Txk (Txk). 48 h after transfection, the Jurkat cells were analyzed for Txk expression (molecular mass, 64 kD) by the immunoblotting method (28). (b) 48 h after transfection, Jurkat and Raji cells were stimulated with PHA plus PMA and PMA plus ionomycin, respectively. Cytokine production was measured by ELISAs. IL-4 production was not detected in any of the transfected Jurkat cell cultures. Mean of triplicate cultures is shown. SEM never exceeded 10% of the mean and was thus omitted. Without mitogenic stimulation, neither Jurkat nor Raji cells produced the cytokines tested, so the results were omitted. Results shown are representative of six independent experiments. ND, not detected. (c) Intracytoplasmic IFN- γ protein expression was assessed by immunofluorescence analysis with anti-IFN- γ mAb of the Jurkat cells. Txk- and mock-transfected Jurkat cells were cultured for 48 h to induce Txk expression. The transfected cells were stimulated with PHA plus PMA for 8 h or kept unstimulated and then analyzed for intracytoplasmic IFN- γ expression. (d) Limiting dilution RT-PCR analysis of IFN- γ mRNA expression. The transfected Jurkat T cells were stimulated with PHA plus PMA for 6 h. We used limiting dilution RT-PCR technique, where varying dilutions of cDNAs were subjected to PCR amplification to more precisely determine effects of Txk transfection on IFN- γ mRNA expression. Lanes 1, 2, and 3 represent 10, 5, and 2% of the total cDNA used for PCR amplification, respectively. Other PCR reaction conditions were exactly the same for lanes 1, 2, and 3. This was to show that PCR reaction ranges within the logarithmic phase of the PCR amplification. (e) Luciferase assay of the Jurkat cells. Jurkat cells were cotransfected with pIFN- γ (-538)-luciferase, pRSV-CAT, and pME18S-Txk or pME18S. 48 h after transfection, half of the cells were stimulated with PHA plus PMA for 8 h, and the remaining cells were kept unstimulated. pRSV-CAT (for pIFN- γ promoter-luciferase and pIL-2 promoter-luciferase) and pGL-3 control (for pIL-4 promoter-CAT) were used for estimating the transfection efficiency. According to the transfection efficiency, the luciferase and CAT activities of the promoter assays were corrected.

mic IFN- γ -positive cells (Fig. 2 c). Txk transfection did not affect IL-2 production of the Jurkat cells (Fig. 2 b). Txk-transfected and mock-transfected Jurkat cells did not produce detectable levels of IL-4 upon stimulation (Fig. 2 b). Txk transfection of Raji cells did not induce IFN- γ , IL-2, or IL-4 production, even upon stimulation with PMA plus ionomycin (Fig. 2 b). These results suggest that Txk has a key role in IFN- γ production by T cells.

We next studied the IFN- γ mRNA expression of the Jurkat cells. Txk transfection led to enhanced IFN- γ mRNA expression by the Jurkat cells as compared with the

mock transfection (Fig. 2 d). We also examined whether Txk transfection positively affects IFN- γ gene transcription by enhancing IFN- γ promoter/enhancer activity. To this end, we cotransfected pIFN- γ (-538)-luciferase and pME18S-Txk into Jurkat cells. We found that Txk transfection induced several-fold more luciferase activity in the cells than the mock (pIFN- γ [-538]-luciferase and pME18S)-transfected cells (Fig. 2 e). As control cytokine-promoter plasmids, we used pIL-2(-568)-luciferase and pIL-4(-265)-CAT. We found that Txk transfection did not affect activities of pIL-2 promoter-luciferase- and pIL-4

promoter-CAT-transfected Jurkat cells, regardless of the presence or absence of mitogenic stimulation. The results revealed that Txk acts specifically on IFN- γ promoter/enhancer (-538) and upregulates IFN- γ gene transcription.

Because Txk has a hypothetical nuclear localization signal sequence (16, 17, 29), we examined nuclear translocation of the Txk protein in response to activation signals. Jurkat cells were stimulated with either PHA or IL-12, and subsequent localization of Txk was assessed by immunocytochemical staining (Fig. 3 a). Unstimulated Jurkat cells showed cytoplasmic localization of Txk. Txk protein accumulated in the nuclei of Jurkat cells after treatment for 1 h with PHA. The nuclear accumulation of Txk was specific for PHA, because Txk protein remained in the cytoplasm of Jurkat cells treated with IL-12. The results suggest that Txk itself translocates into nuclei and enhances IFN- γ gene transcription in T cells.

To study the role of nuclear translocation of the Txk protein upon activation, we constructed a pME18S-mutant Txk vector expressing Txk protein that lacked a nuclear lo-

calization signal sequence (KRKP-deleted) (16, 17, 29). Jurkat cells were transfected with either wild-type or mutant Txk expression vector and cultured for 48 h. In the mutant Txk-transfected Jurkat cells, a vast majority of the (mutant) Txk protein stayed in cytoplasm and did not translocate into nuclei, even after stimulation with PHA plus PMA. However, very small amounts of endogenous Txk in the Jurkat cells translocated into nuclei upon activation. In contrast, in the wild-type Txk-transfected Jurkat cells, Txk protein translocated into nuclei in response to the stimulation (Fig. 3 b).

We measured IFN- γ production of the transfected Jurkat cells in parallel experiments. We found that wild-type Txk did enhance IFN- γ production, whereas nuclear localization signal sequence (KRKP)-deleted Txk did not affect IFN- γ production by the transfected Jurkat cells. These results indicate that nuclear localization of Txk is obligatory for its effect on cytokine expression (Fig. 3 c).

To confirm the involvement of Txk in IFN- γ production by human T cells, we tested inhibition of IFN- γ pro-

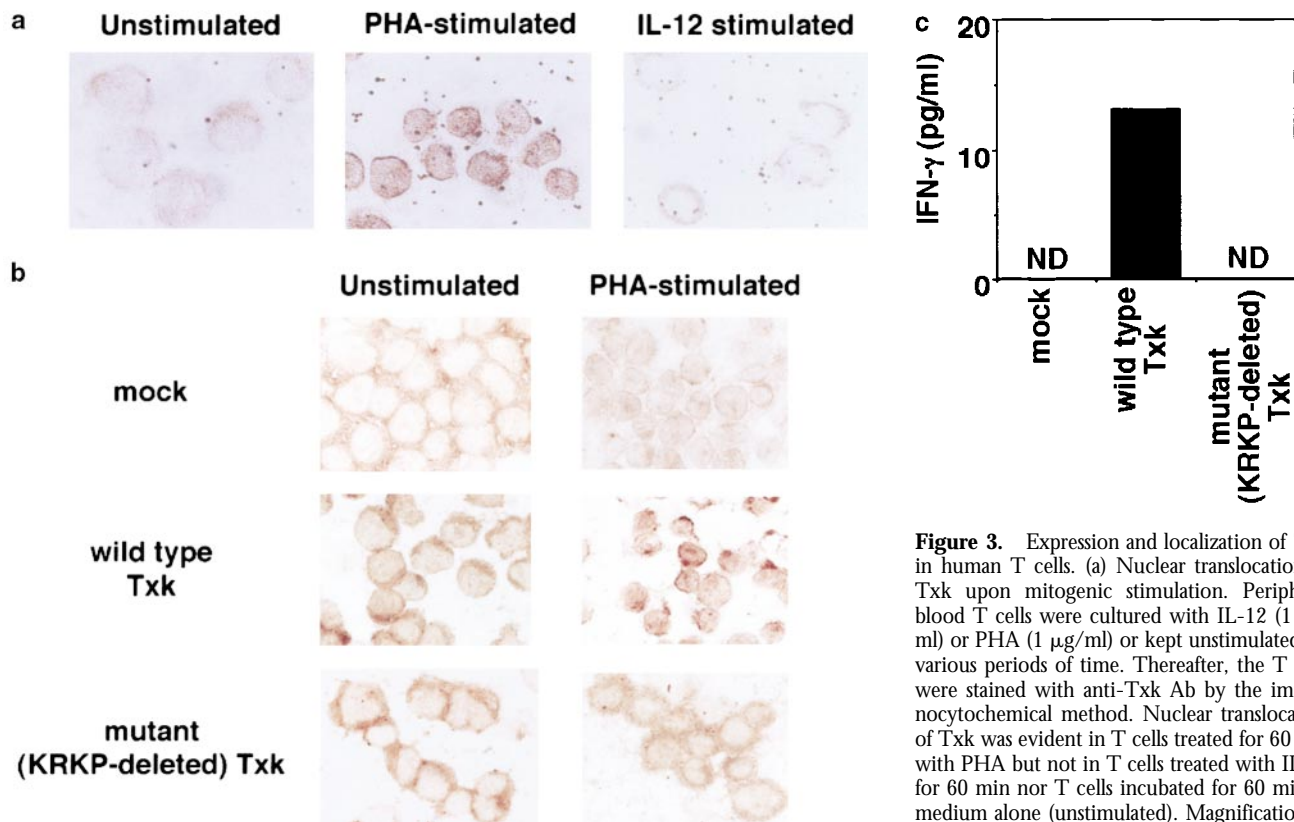


Figure 3. Expression and localization of Txk in human T cells. (a) Nuclear translocation of Txk upon mitogenic stimulation. Peripheral blood T cells were cultured with IL-12 (1 ng/ml) or PHA (1 μ g/ml) or kept unstimulated for various periods of time. Thereafter, the T cells were stained with anti-Txk Ab by the immunocytochemical method. Nuclear translocation of Txk was evident in T cells treated for 60 min with PHA but not in T cells treated with IL-12 for 60 min nor T cells incubated for 60 min in medium alone (unstimulated). Magnification of the results was 250. (b) Immunocytochemical

staining of wild-type Txk- and nuclear localization sequence-deleted mutant Txk-transfected Jurkat cells. Jurkat cells were transfected with pME18S (mock), pME18S-Txk (wild type Txk), or pME18S-mutant (KRKP-deleted) Txk expression vector and cultured for 48 h. Thereafter, the cells were activated with PHA plus PMA for 1 h. Respective cytospin preparations were made and stained with anti-Txk Ab. Control Ab did not stain at all, so results of the control staining were omitted. The mutant Txk did not translocate into nuclei even upon activation. The results shown (magnification 250) are representative of four independent experiments with essentially the same results. (c) Effects of nuclear localization sequence-deleted mutant Txk transfection on IFN- γ production. Jurkat cells were transfected with pME18S (mock), pME18S-Txk (wild type), or mutant (KRKP-deleted) Txk expression vector and cultured for 48 h. Thereafter, the cells were activated with PHA plus PMA for 24 h. IFN- γ production by the transfected Jurkat cells was assessed by ELISA. The results shown are representative of four independent experiments with essentially the same results.

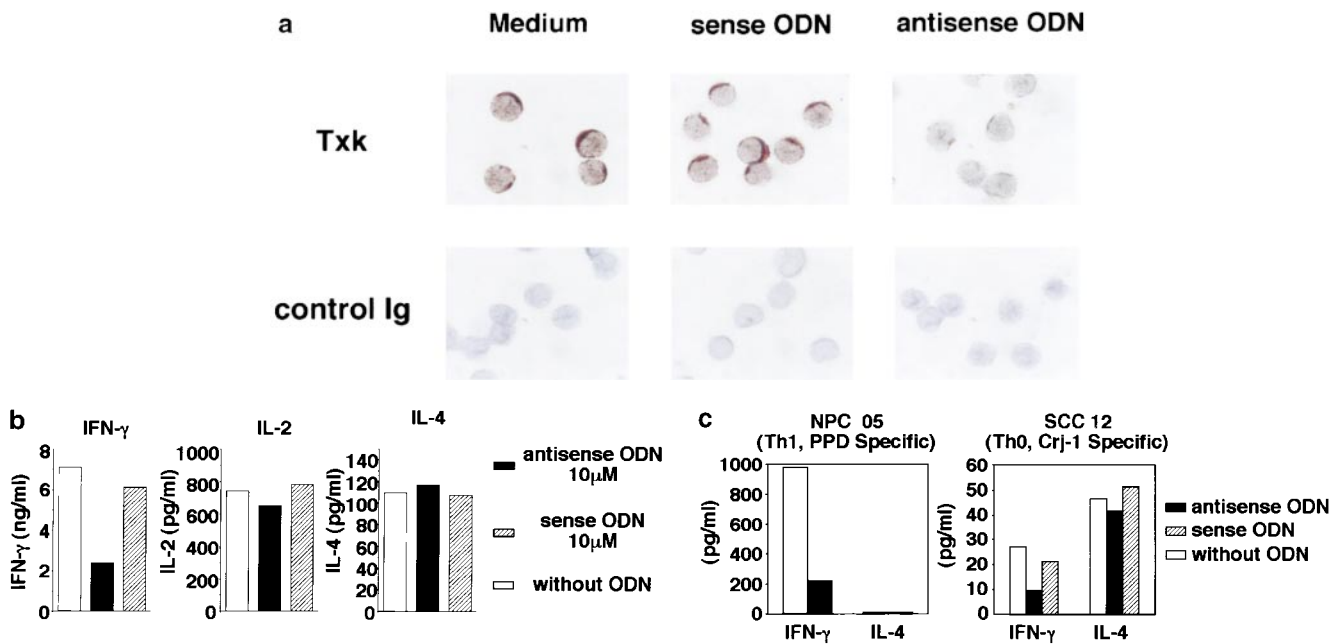


Figure 4. Effects of Txk antisense ODN on cytokine production by human T cells. (a) Txk expression of normal peripheral blood T cells treated with Txk antisense ODN was analyzed. Txk expression was specifically reduced when treated with Txk antisense ODN, but not sense ODN (both 10 μ M), for 12 h. The result shown (magnification 250) is representative of three independent experiments with essentially the same results. (b) Normal peripheral blood T cells were cultured in the presence of Txk antisense ODN for 12 h to reduce Txk expression. Thereafter, the T cells were stimulated with PHA. The result shown is representative of three independent experiments with essentially the same results. Mean of triplicate cultures is shown. SEM never exceeded 10% of the mean and was thus omitted. (c) PPD- and Crj-1-specific T cell clones pretreated with the ODNs (5 μ M, which inhibits Txk expression of the clones) were stimulated with the relevant Ag plus irradiated autologous PBMCs. The similar results were reproduced in the experiments using cells from different donors and different Ag-specific T cell clones. Mean of triplicate cultures is shown. SEM never exceeded 10% of the mean and was thus omitted.

duction by Txk antisense ODN. Peripheral blood T lymphocytes were cultured in the presence of sense or antisense ODN corresponding to the original translation start site of Txk (16) for several hours and were then stimulated with PHA. Antisense ODN, but not sense ODN, specifically inhibited cytoplasmic expression of Txk (Fig. 4 a); IFN- γ production of T cells was specifically inhibited by the antisense ODN (Fig. 4 b). IL-2 and IL-4 production were not modulated by either the sense or antisense ODNs (Fig. 4 b). To further confirm that Txk antisense ODN inhibits IFN- γ production, Ag-specific T cell clones were cultured with ODNs and then stimulated with Ag plus irradiated autologous PBMCs. Again, IFN- γ production by the Ag-specific Th1 and Th0 clones (Fig. 4 c), but not IL-4 production by the Ag-specific Th0 (Fig. 4 c) and Th2 clones (data not shown) was inhibited by the Txk antisense ODN.

We also studied whether Txk expression of human T cells is under the influence of Th1 cytokines. Normal peripheral blood CD4⁺ T cells were cultured with various concentrations of Th1 and Th2 cytokines. Intracytoplasmic Txk protein in T cells was subsequently assessed by flow cytometric analysis with anti-Txk Ab (Fig. 5). IL-2 treatment of the CD4⁺ T cells did not affect Txk expression levels. IL-4 markedly reduced Txk expression of the CD4⁺ T cells (Fig. 5). In contrast, IL-12 treatment for 4 h was

sufficient to enhance Txk expression of the CD4⁺ T cells (Fig. 5). This further supports an intimate association between Th1 cells and Txk expression. IL-12 may be involved in the polarization toward Th1 cells of T cells via Txk but is not affected by the outcome of IFN- γ production via Txk.

It has been shown that Txk protein includes two isoforms that arise by alternative initiation of translation from the same cDNA (16). We confirmed that Txk protein has two isoforms in COS cells when overexpressed by transfection (data not shown). However, Jurkat cells and normal PBLs almost exclusively express a longer isoform of Txk

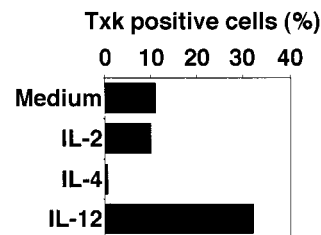


Figure 5. Effects of cytokine treatment on Txk expression. Purified peripheral blood CD4⁺ T cells were cultured with various concentrations of recombinant cytokines for 4 h. Thereafter, intracytoplasmic Txk expression of the T cells was analyzed by immunofluorescence staining using anti-Txk Ab. IL-12 (1 ng/ml) enhanced whereas IL-4 (10 ng/ml) reduced Txk expression of the T cells. IL-2 (2 ng/ml) treatment did not significantly affect Txk expression. The results shown are representative of five independent experiments with essentially the same results.

(Fig. 2 a). In normal PBLs, antisense ODN, which primes with the original translation start site (the longer isoform), almost completely abolished Txk staining (Fig. 4 a), suggesting that a longer isoform of Txk mainly mediates regulation of IFN- γ production in T cells.

In summary, Txk expression is restricted to Th1/Th0 cells with IFN- γ producing potential and is significantly involved in IFN- γ gene transcription and subsequent IFN- γ protein production in human T cells.

We believe that this is the first description of Txk involvement in IFN- γ production by human Th1 cells. More recently, we have found that Txk is phosphorylated and translocates into nuclei upon activation, and Txk or a protein complex including Txk binds to the IFN- γ promoter sequence (Nagafuchi, H., N. Suzuki, and T. Sakane, unpublished observation). Thus, we are currently investigating whether Txk itself or a protein complex including Txk acts as a Th1 cell-specific transcription factor for the IFN- γ gene.

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