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

T helper 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 important 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)2 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 acetyltransferase; ODN, oligodeoxynucleotide; p, plasmid; RSV, Rous sarcoma virus; RT, reverse transcription; SH, src homology.
Materials and Methods

Establishment of A g-specific T Cell Clones and Cytokine Production. House dust mite-specific, Japanese cedar pollen Cryptome-ria 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 T CR-αβ, CD3, CD4, and CD45R O 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 human Txk cDNA was amplified by PCR using a sense primer (CGGAATTCATGACTTCTGTCGTCTCTGATCTAAACA) and an antisense primer (TTCCTCTAGATGACCCATTCTTTCGCAATTCG), 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 Pfu Turbo DNA polymerase (Stratagene Inc.). The amplification cycle consisted of 1 cycle of denaturation (95°C) for 1 min, followed by 15 cycles of denaturation (95°C) for 30 s, annealing for 1 min (55°C), and polymerization for 1 min (68°C). After PCR cycling, the PCR product was treated with Dpn I, 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 E. coli DH5α.

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'-CGGCGCCGTGTCGAGCGCGTACTGCTCCTCC-TCCCCACCTCT-3' and 5'-GAGGTCGAGGGAGGAAGGGCGAGTGACGCGTCAGCAAGCGCCCCG-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 μg/ml) plus PMA (10 ng/ml) or PMA (10 ng/ml) plus ionomycin (1 μg/ml) for 24 h to induce lymphokine production. 5 μg of plasmid (pIFNy-γ (−538)-luciferase (provided by Dr. C.B. Wilson, University of Washington, Seattle, WA), 5 μg pRSV (Roche Diagnostics) were similarly transfected into Jurkat cells.

Antibodies. T cell 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). Fluorescein isothiocyanate-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 0.1 M Hepes buffer solution. T hereafter, intracytoplasmic antibodies were stained with purified first Abs. Biotin-conjugated second Abs and streptavidin–fluorochrome. T hereafter, 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 cytokine 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, TTGTCTCTCACTGACGAGA; Txk antisense, GCA-CCCTCTTCTGACTCCT; 475-bp product.

Antisense 5′ oligodeoxynucleotide and Cell Culture. Sense (GGG-CTACCATGAGGTTTGC) and antisense (GAAACCTCATGG-
TAGCCC) oligodeoxynucleotides (ODNs) specific for Txk were synthesized as a sulfonylated form. Peripheral blood T cells and Ag-specific cloned T cells (10^6 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 Txk 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 Txk. Both peripheral blood CD4^+ and CD8^+ T cells expressed Txk; peripheral blood B cells and monocytes, however, never expressed it. We next asked whether Txk 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 Txk 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 Txk; all of these clones have IFN-γ producing potential (Fig. 1, a and b). However, Th2 clones, regardless of Ag specificity or the donor, did not express Txk at all (Fig. 1, a and b). Thus, there is an intimate association between Txk expression and Th1/Th0 clones with IFN-γ producing potential.

We next studied the effects of Txk overexpression on cytokine production by T cells. To this end, Jurkat cells were transfected with pME18S-Txk. We used Jurkat cells in these experiments because they produce relatively low levels of Th1 cytokines upon mitogen activation. Overexpression of Txk by the transfection was confirmed by immunoblotting with anti-Txk Ab (Fig. 2 a). In parallel experiments, Jurkat cells were stimulated with PHA plus PMA. When we used ELISA to detect cytokine secretion, Txk transfection resulted in several-fold more IFN-γ production as compared with the mock transfection (Fig. 2 b). We also confirmed that Txk 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, Txk 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 Txk-transfected Jurkat cells stimulated with PHA plus PMA, 36% were intracytoplasmic IFN-γ-positive cells; in mock-transfected Jurkat cells stimulated with PHA plus PMA, 13% were intracytoplasmic IFN-γ-positive cells; in Txk-transfected Jurkat cells stimulated with PHA plus PMA, 36% were intracytoplasmic IFN-γ-positive cells.
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, 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.
promoter-CAT–transfected Jurkat cells, regardless of the presence or absence of mitogenic stimulation. The results revealed that T\(xk\) acts specifically on IFN-\(\gamma\) promoter/enhancer (-538) and upregulates IFN-\(\gamma\) gene transcription.

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

To study the role of nuclear translocation of the T\(xk\) protein upon activation, we constructed a pME18S-mutant T\(xk\) vector expressing T\(xk\) protein that lacked a nuclear localization signal sequence (KRKP-deleted) (16, 17, 29). Jurkat cells were transfected with either wild-type or mutant T\(xk\) expression vector and cultured for 48 h. In the mutant T\(xk\)-transfected Jurkat cells, a vast majority of the (mutant) T\(xk\) protein stayed in cytoplasm and did not translocate into nuclei, even after stimulation with PHA plus PMA. However, very small amounts of endogenous T\(xk\) in the Jurkat cells translocated into nuclei upon activation. In contrast, in the wild-type T\(xk\)-transfected Jurkat cells, T\(xk\) protein translocated into nuclei in response to the stimulation (Fig. 3 b).

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

To confirm the involvement of T\(xk\) in IFN-\(\gamma\) production by human T cells, we tested inhibition of IFN-\(\gamma\) production by human T cells, we tested inhibition of IFN-\(\gamma\) production by human T cells, we tested inhibition of IFN-\(\gamma\) production by human T cells, we tested inhibition of IFN-\(\gamma\) production by human T cells, we tested inhibition of IFN-\(\gamma\) production by human T cells, we tested inhibition of IFN-\(\gamma\) production by human T cells, we tested inhibition of IFN-\(\gamma\) production by human T cells.
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production 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. 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. (c) PPD- and Crj-1-specific T cell clones pretreated with the ODNs (5 μM, 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.

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 μM), 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 μM, 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.

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.

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.
(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 (Nagauchi, 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.

We thank Drs. S. Toyoshima, N. Yamashita, and K. Sumita for their helpful discussion. We are also indebted to Ms. E. Takada for her excellent technical assistance.

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Submitted: 5 May 1999 Revised: 19 July 1999 Accepted: 20 July 1999

References

1. Mosmann, T.R., and R.L. Coffman. 1989. TH1 and TH2 cells different patterns of lymphokine secretion lead to different functional properties. Ann. Rev. Immunol. 7:145–173.
2. Seder, R.A., and W.E. Paul. 1994. Acquisition of lymphokine-producing phenotype by CD4+ T cells. Ann. Rev. Immunol. 12:635–673.
3. Gately, M.K., L.M. Renzetti, J. Magram, A.S. Stern, L. Adorini, U. Gubler, and D.H. Presky. 1998. The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. Ann. Rev. Immunol. 16: 495–521.
4. Ouyang, W., S.H. Ranganathan, K. Weindel, D. Bhattacharya, T.L. Murphy, W.C. Sha, and K.M. Murphy. 1998. Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. Immunity. 9:745–755.
5. Li-Weber, M., P. Salgame, C. Hu, I.V. Davydov, O. Laur, S. Klevenz, and P.H. Krammer. 1998. Altered interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. Hum. Mol. Genet. 7:145–173.
6. Scala, E., M. Carbonari, P. Del Porto, M. Cibati, T. Tedesco, A.M. Mazzone, R. Paganelli, and M. Fiorilli. 1998. Lymphocyte activation gene-3 (LAG-3) expression and IFN-γ production are variably coregulated in different human T lymphocyte subpopulations. J. Immunol. 161:489–493.
7. M urray, J.S. 1998. How the MHC selects Th1/Th2 immunity. Immol. today. 19:157–163.
8. Moriggl, R., C. Kristofic, B. Kinzel, S. Volarevic, B. Groner, and V. Brinkmann. 1998. Activation of STAT proteins and cytokine genes in human Th1 and Th2 cells generated in the absence of IL-12 and IL-4. J. Immunol. 160:3385–3392.
9. Gollob, J.A., E.A. Murphy, S. Mayhan, C.P. Schnipper, J. Ritz, and D.A. Frank. 1998. Altered interleukin-12 responsiveness in Th1 and Th2 cells is associated with the differential activation of STAT5 and STAT1. Blood. 91:1341–1354.
10. Hu, Q., D. Davison, P.L. Schwartzberg, F. M acciarini, M.J. Lenardo, J.A. Bluestone, and L.A. Matis. 1995. Identification of Rlk, a novel protein tyrosine kinase with predominant expression in the T cell lineage. J. Biol. Chem. 270: 1928–1934.
11. Haire, R.N., Y. O hta, J.E. Lewis, S.M. Fu, P. Kroisel, and G.W. Litman. 1994. TXK, a novel human tyrosine kinase expressed in T cells shares sequence identity with T ec family kinases and maps to 4p12. Hum. Mol. Genet. 3:897–901.
12. Sommers, C.L., K. Huang, E.W. Shores, A. Grinberg, D.A. Charlick, C.A. Kozak, and P.E. Love. 1995. Murine txk: a protein tyrosine kinase gene regulated by T cell activation. O nco gene. 11:245–251.
13. O hta, Y., R.N. Haire, C.T. Amemiya, R.T. Litman, T. Trager, O. Riess, and G.W. Litman. 1996. Human TXK: a genome organization, structure and contiguous physical linkage with the T ec gene. O ngene. 12:937–942.
14. Ellis, J.H., R.P. Sutmuller, M.J. Sims, and S. Cooksey. 1998. Functional analysis of the T-cell-restricted protein tyrosine kinase T Xk. Biochem. J. 335:277–284.
15. Schneider, H., P.L. Schwartzberg, and C.E. Rudd. 1998. Resting lymphocyte kinase (Rlk/Txk) phosphorylates the YVKM motif and regulates PI-3-kinase binding to T-cell antigen CTLA-4. Biochem. Biophys. Res. Commun. 252:14–19.
16. Debnath, J., M. Chamorro, M.J. Czar, E.M. Schaefier, M.J. Lenardo, H.E. Varmus, and P.L. Schwartzberg. 1999. rlk/T XK encodes two forms of a novel cysteine string tyrosine kinase activated by Src family kinases. M ol. C ell. B iol. 19: 1498–1507.
17. M ano, H. 1999. The T ec family protein-tyrosine kinases: a novel signal for kinases of a subset of signalings. Int. J. Hematol. 69: 6–12.
18. Vetrie, D., I. Vorechovsky, P. Sideras, J. Holland, A. Davies, F. Flinter, L. H ammarstrom, C. Kin nom, R. Levinsky, M. Bobrow, et al. 1993. The gene involved in X-linked agamaglobulinemia is a member of the src family of protein-tyrosine kinases. N ature. 361:226–233.
19. Siliciano, J.D., T.A. Morrow, and S.V. Desiderio. 1992. Itk, a T-cell-specific tyrosine kinase gene inducible by interleukin 2. Proc. Natl. Acad. Sci. U.S.A. 89:11194–11198.

20. Tamagnone, L., I. Lahtinen, T. Mustonen, K. Virtaneva, F. Francis, F. Muscatelli, R. Alitalo, C.I. Smith, C. Larsson, and K. Alitalo. 1994. BMX, a novel nonreceptor tyrosine kinase gene of the BTK/ITK/TEC/TXK family located in chromosome Xp22.2. Oncogene. 9:3679–3688.

21. Salim, K., M.J. Bottomley, E. Querfurth, M.J. Zvelebil, I. Gout, R. Scafe, R.L. Margolis, R. Gigg, C.I. Smith, P.C. Driscoll, et al. 1996. Distinct specificity in the recognition of phosphoinositides by the pleckstrin homology domains of dynamin and Bruton's tyrosine kinase. EMBO (Eur. Mol. Biol. Organ.) J. 15:6241–6250.

22. August, A., A. Sadra, B. Dupont, and H. Hanafusa. 1997. Src-induced activation of inducible T cell kinase (ITK) requires phosphatidylinositol 3-kinase activity and the pleckstrin homology domain of inducible T cell kinase. Proc. Natl. Acad. Sci. U.S.A. 94:11227–11232.

23. Li, T., S. Tsukada, A. Satterthwaite, M.H. Havlik, H. Park, K. Takatsu, and O.N. Witte. 1993. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. Cell. 72:279–290.

24. Kaneko, S., N. Suzuki, H. Koizumi, S. Yamamoto, and T. Sakane. 1997. Rescue by cytokines of apoptotic cell death induced by IL-2 deprivation of human antigen specific T cell clones. Clin. Exp. Immunol. 109:185–193.

25. Yamashita, N., H. Kaneoka, S. Kaneko, M. Takeno, K. Omeda, H. Koizumi, M. Kogure, G. Inaba, and T. Sakane. 1997. Role of γδ T lymphocytes in the development of Behçet's disease. Clin. Exp. Immunol. 107:241–247.

26. Suzuki, N., M. Ichino, S. Mihara, S. Kaneko, and T. Sakane. 1998. Inhibition of Fas/Fas ligand-mediated apoptotic cell death of lymphocytes in vitro by circulating anti-Fas ligand autoantibodies in patients with systemic lupus erythematosus. Arthritis Rheum. 41:344–353.

27. Robbins, J., S.M. Dilworth, R.A. Laskey, and C. Dingwall. 1991. Two interdependent basic domains in nucleoplasmic nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. Curr. Biol. 1:615–623.

28. Sander, B., J. Andersson, and U. Andersson. 1991. Assessment of cytokines by immunofluorescence and the paraformaldehyde-saponin procedure. Immunol. Rev. 119:65–93.

29. Brenner, C.A., A.W. Tam, P.A. Nelson, E.G. Engleman, N. Suzuki, K.E. Fry, and J.W. Larrick. 1989. Message amplification phenotyping (MAPPing): a technique to simultaneously measure multiple mRNAs from small numbers of cells. Biotechniques. 7:1096–1103.
The authors regret that the sequences of Txk sense and antisense oligodeoxynucleotides (ODNs) given on pp. 1148-1149 were incorrect. The correct sequences are as follows.

Txk sense ODN:        GGGCTACCATGATCCTTTC
Txk antisense ODN:     GAAAGGATCATGGTAGCCC