

Th1-dominant shift of T cell cytokine production, and subsequent reduction of serum immunoglobulin E response by administration *in vivo* of plasmid expressing Txk/Rlk, a member of Tec family tyrosine kinases, in a mouse model

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Summary

Background Th1 and Th2 cells, resulting from antigenic stimulation in the presence of IL-12 and IL-4, respectively, are implicated in the pathology of various diseases including allergic and autoimmune diseases. Txk/Rlk is a member of Tec family tyrosine kinases. We reported that Txk acts as a Th1-specific transcription factor in the T lymphocytes.

Objective In this study we have asked whether administration of txk expression plasmid brings about a Th1/Th2 shift *in vivo* of the mice, and subsequent reduction of circulating IgE.

Methods Mice were administered a txk expression plasmid with hemagglutinating virus of Japan (HVJ) envelope vector. Txk expressions in spleen cells were assessed by immunoblotting and immunocytochemical staining. Cytokine productions by the spleen cells and serum Ig concentrations were studied by ELISA.

Results Administration of a txk expression plasmid with HVJ vector induced expression of Txk in the spleen cells. The spleen cells showed enhanced Th1-specific cytokine production; spleen cells from the txk administered mice produced more IFN- γ as compared with those from control plasmid-administered mice in an antigen-specific manner. IL-2 and IL-4 secretions of the spleen cells were comparable between the two mouse groups. Txk administration did not reduce serum IgG concentration. It markedly reduced total IgE level and an IgG1/IgG2a ratio, reflection of Th1/Th2 balance, in sera. Furthermore, txk administration reduced ovalbumin (OVA)-specific IgE levels in sera of the OVA sensitized mice.

Conclusion Thus, Txk enhances IFN- γ secretion and thus modulates Th1/Th2 cytokine balance, leading to reduction of serum IgE.

Keywords allergy, cytokines, gene therapy, IgE, kinase, Th1/Th2 cells, Txk/Rlk

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Introduction

Th1 and Th2 cells produce distinct sets of cytokines and dictate distinct immune responses. Th1 cells produce IFN- γ and IL-2, and promote effective immune responses against intracellular pathogens, although they have also been implicated in autoimmune pathologies. Th2 cells produce IL-4, IL-5, and IL-13 and eradicate extracellular parasites but also mediate allergic and atopic manifestations [1–5].

Development of an appropriate Th response to a pathogen is critical for the outcome of a protective immune response [3–5]. Cytokines present at the initiation of CD4⁺ T cell responses predominantly determine the development of a particular Th subtype. IL-12 and IL-4 direct antigen-

stimulated naïve CD4⁺ T cells toward Th1 and Th2 development, respectively [6, 7]. Development of committed Th1 and Th2 phenotypes displaying an exclusive cytokine production pattern is achieved largely after repeated antigenic stimulation under appropriate conditions. Although cytokine signals may modulate the phenotypes of Th1 and Th2 populations at an early stage of development, these subsets become irreversible after repeated stimulation [8–10].

Evidence indicates that IL-4-induced Th2 development rapidly blocks IL-12 signalling by extinguishing IL-12R β 2 expression [11, 12]. Conversely, a selective defect in IL-4 signalling in committed Th1 cells has also been reported [13]. Recent efforts have defined several transcription factors that are differentially expressed in Th1 and Th2 cells; GATA-3 and c-Maf in Th2 cells [14, 15]; ERM and T-bet in Th1 cells [16, 17]. It has been shown that forced expression of GATA-3 gives ability of Th2 cytokine production on Th1 cells *in vitro* [15]. These subtype-specific transcription factors may be involved in Th1- and Th2-specific cytokine expression by directly activat-

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ing cytokine gene promoters *in vitro* [14, 16, 18, 19]. Thus, it is interesting to test whether modulation from Th2 dominant to Th1 dominant immune responses is achievable by artificial gene transfer of these transcription factors *in vivo*.

Txk/Rlk is a member of Tec family tyrosine kinases involved in the T cell activation signal pathway [20–28]. We found recently that Txk acted as Th1-specific transcription factor, in co-operation with a factor(s) induced upon T cell receptor signalling, and governed Th1-specific expression of the IFN- γ gene by interacting directly with a critical regulatory element of its promoter [29–31].

We therefore determined the effect of artificial txk expression on Th2 dominant immune responses using a plasmid that produces txk. We found that administration of the txk plasmid resulted in expression of IFN- γ in spleen cells, leading to the Th1/Th2 shift of the spleen cells and reduction of circulating IgE.

Materials and methods

Mice and immunization

Specific pathogen-free, 6-week-old female BALB/c mice obtained from Japan Clea (Kawasaki, Japan) were used in all studies. Animal use adhered to our institutional laboratory animal care guidelines. The mice were administered the plasmid (50 μ g) by intravenous injection (tail vein) with hemagglutinating virus of Japan (HVJ) envelope vector three times (days –1, +1, and +3) [32]. Thereafter, the mice were immunized intraperitoneally with 50 μ g ovalbumin (OVA; Wako, Osaka, Japan) at day 0. As an adjuvant, either complete Freund's adjuvant (CFA; Difco Laboratories, Detroit, MI, USA) or alum (LSL Co., Tokyo, Japan) was used. Three weeks later, the same plasmid (50 μ g) was given three times (days +20, +22, and +24). Booster immunization with OVA and incomplete Freund's adjuvant (ICFA; Difco Laboratories) or alum was carried out at day +21. In some experiments, purified *Cryptomeria japonica* (Cry J)1 antigen, a major allergen of Japanese cedar (Cry J) pollen was used (Fig. 1) [33]. The weight of each mouse was monitored every 24 h, and mice were killed at the indicated time-points for assessment of Txk expression and cytokine production (Fig. 1).

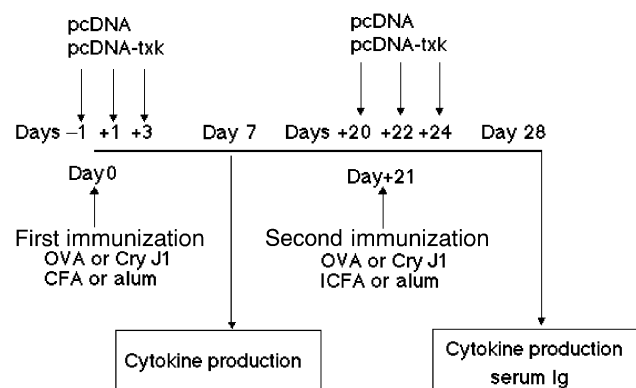


Fig. 1. Immunization scheduler of the experiment. BALB/c mice were immunized with ovalbumin (OVA) or *Cryptomeria japonica* (Cry J)1. pcDNA vector expressing mouse Txk or empty pcDNA vector was given to the mice. In some experiments, the mice were killed at day 7. The remaining mice were killed at day 28.

Western blotting analysis

Fifty micrograms of cellular extracts from spleen cells was separated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore, Tokyo, Japan) [30]. The immunoblotting was performed with an anti-Myc-tag antibody (Invitrogen, Carlsbad, CA, USA), followed by a horseradish peroxidase-coupled secondary antibody (Amersham Bioscience, Tokyo, Japan), and visualized using a chemiluminescence substrate (Pierce Biotechnology, Rockford, IL, USA). As a protein loading control, anti-actin (Neomarkers, Fremont, CA, USA) immunoblotting was conducted.

Plasmids

Mouse txk cDNA expression vector (pcDNA-mouse txk/rlk) was kindly provided by Dr J. Debnath (NCI, NIH, Bethesda, MD, USA) [23–25]. The vector contained cytomegalovirus (CMV) promoter and c-myc-tag at its 3' end. As a control vector, we used pcDNA3.1 (Invitrogen). A large quantity of purified plasmid was prepared by two cycles of CsCl₂ ultracentrifugation. The purified plasmid was kept at –70 °C until use.

Immunocytochemical staining

Spleen cells 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 the first antibody overnight at 4 °C. All subsequent procedures were performed using LSAB2/AP kit (DAKO JAPAN, Kyoto, Japan). The home-made rabbit anti-Txk antibody was developed using recombinant whole Txk expressed by *E. coli*, and used as an affinity purified antibody [29–31].

Cytokine and Ig assays

The cell culture medium was RPMI 1640 medium supplemented with 10% FCS, 2 mM glutamine, 100 U/mL penicillin, 100 μ g/mL streptomycin, 50 μ M 2-ME, 10 mM HEPES buffer, and 1 mM sodium pyruvate. The spleen cells (1×10^6 /mL) were stimulated with Concanavalin A (Con A; 1 μ g/mL, Amersham Bioscience) or the immunizing antigen, OVA (10 μ g/mL) or Cry J1 (5 μ g/mL) [33] for 24 h. As an irrelevant antigen, human γ -globulin (HGG; Sigma, St Louis, MO, USA) or keyhole limpet hemocyanin (KLH; Calbiochem, Darmstadt, Germany) was used. The supernatants were collected and assayed for cytokine content (IFN- γ , IL-2, IL-4) by commercial ELISA (for IL-2 and IL-4, Pharmingen, San Diego, CA, USA; for IFN- γ , Biosource International, Camarillo, CA, USA).

Serum IL-12 concentration was also determined by commercial ELISA (Pharmingen). Serum IgG, IgG1, IgG2a, and IgE were measured by using mouse Ig ELISA quantitation kits (Bethyl Laboratories, Montgomery, TX, USA).

A reverse, or IgE-capture, ELISA was used for measuring OVA-specific IgE antibody in the serum of immunized mice [34]. Microplate wells were first coated with a commercial anti-mouse IgE rat monoclonal antibody, and then incubated with twofold serial dilutions of test sera with 10% normal

mouse serum as diluent for the capturing of only IgE class molecules. Biotinylated OVA and then beta-D-galactosidase-conjugated streptavidin were added and, finally, 4-methylumbelliferyl-beta-D-galactoside was used as the enzyme substrate. The fluorescence intensity of the reaction product (4-methylumbelliferone) was determined on a microplate fluorescence reader. The sensitivity of this assay was equal to that of passive cutaneous anaphylaxis (PCA). In contrast to indirect ELISAs, this IgE-capture assay is free from competition by non-IgE antibodies. Furthermore, it requires much less antigen than the PCA assay.

Statistical analysis

Results of irregular distribution in the experiments were compared by Mann-Whitney *U*-test. Where appropriate, we used Student's *t*-test instead of *U*-test. A *P*-value less than 0.05 was judged to be statistically significant.

Results and discussion

In initial studies, we determined the ability of a mouse *txk* expression plasmid to express Txk in spleen cells of the mice. Intravenous administration of *txk* expression plasmid with HVJ envelope vector did not itself cause weight loss or other harmful effects of the mice (data not shown). We studied *txk* mRNA expression by RT-PCR method. We found that administration of a control plasmid (empty pcDNA) did not induce enhanced expression of *txk* mRNA in the spleen. The plasmid-derived *txk* mRNA was expressed in the spleen cells of mice receiving *txk* expression plasmid (data not shown). We then conducted immunoblotting analysis with anti-Myc antibody, because the *txk* expression plasmid contained myc-tag at its 3' end. We found that myc-tag was detected in the spleen cells of mice administered *txk* expression plasmid (Fig. 2a). We next performed immunostaining of Txk in the spleen cells. Spleen cells from control plasmid-injected mice expressed low levels of Txk, which was probably reflection of endogenous Txk; however, the percentage of Txk⁺ cells was low (2–3%) (Figs 2b and c). In contrast, ~20% of the spleen cells expressed Txk protein in the mice given *txk* expression plasmid (Figs 2b and c). HVJ envelope vector was constructed by incorporating plasmid DNA into inactivated HVJ particles. Kaneda et al. reported that intravenous injection of HVJ vector with an exogenous gene led to preferential expression of the gene in the spleen [32]. We next studied whether CD4⁺ T cells express Txk. To this end, CD4⁺ cells were negatively selected by autoMacs system [35], and Txk expression was analysed. We found that 10–20% of the CD4⁺ T cells express Txk. Taken together, these results showed that *txk* expression plasmid was taken up and Txk was expressed in CD4⁺ T cells of normal mouse spleen.

We then determined the effects of administration of *txk* expression plasmid on cytokine production by the spleen cells after first immunization. In the studies, spleens were obtained from mice that were administered *txk* expression plasmid or control plasmid at day 7 (Fig. 1). After cell culture with a relevant antigen, an irrelevant antigen or Con A, IL-2, IL-4, and IFN- γ levels were measured by ELISA. OVA, an immunizing antigen, stimulated spleen cells obtained from

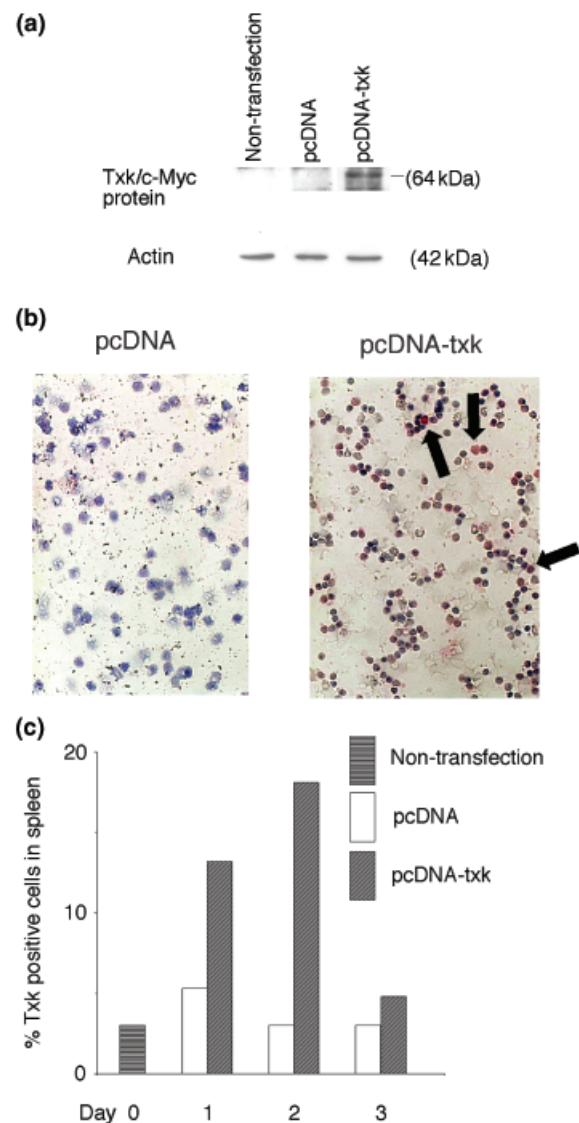


Fig. 2. Detection of Txk protein expression in spleen cells from mice injected txk expression vector. (a) Expression of the vector-derived c-Myc tag in the spleen cells administered txk expression vector. Normal mice were administered Txk expression vector for the first time. The spleen cells were recovered 2 days after the administration and c-Myc expression was analysed by immunoblotting. As a protein loading control, anti-actin immunoblotting was conducted. Data shown were representative of three independent experiments with similar results. (b) Cytopsin preparation of the spleen cells was made. The spleen cells were immunostained with the anti-Txk antibody. Arrows indicate some of the Txk protein expressing cells. Control IgG did not stain at all, thus was omitted. Data shown were representative of four independent experiments with similar results. (c) Percent Txk positive cells were calculated on the cytopsin preparation of the spleen cells. We counted more than 500 cells/sample. Results shown were representative of four independent experiments with similar results.

mice administered *txk* expression plasmid produced consistently increased amounts of IFN- γ *in vitro*, as compared with cells obtained from mice administered control plasmid (Fig. 3). Stimulation with an irrelevant antigen such as HGG did not provoke detectable levels of cytokine production (data not shown). IL-2 production was comparable between the two groups of mice. However, in some experiments, IL-2 production by the spleen cells was enhanced. This may be due

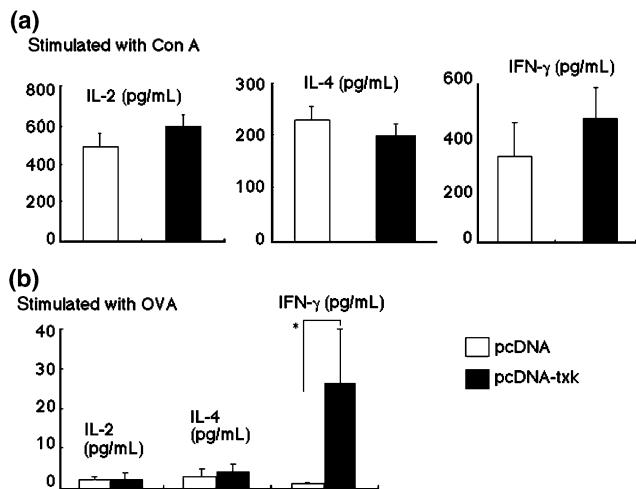


Fig. 3. *Ex vivo* cytokine production of spleen cells from mice given the txk expression plasmid: analysis after first immunization. Effects of intravenous injection of txk expression plasmid on cytokine production by spleen cells immunized with ovalbumin (OVA). First immunization with OVA+complete Freund's adjuvant (CFA) was conducted at day 0. At day 7, spleens of the 10 mice were recovered (please see Fig. 1). The spleen cells were stimulated with Concanavalin A (Con A) (a) or an immunizing antigen, OVA (b) for 24 h. The 24 h-culture supernatants were recovered and assayed for the cytokine production. The irrelevant antigen, human γ -globulin (HGG) did not induce detectable cytokine production, thus the results were omitted. Results shown were mean+SD of 10 mice, and were representative of six independent experiments. * $P < 0.05$ by Mann-Whitney *U*-test.

to secondary event of txk administration; after enhanced IFN- γ production by Txk, differentiation of naïve Th cells into Th1 cells may have become dominant, and as a result, IL-2 production by the cells was up-regulated. However, IL-4 production that we initially expected to be reduced in the mice administered txk expression plasmid, was almost comparable between the two groups of mice. However, when we used Con A as a polyclonal activator of the T cells, the enhancement of IFN- γ was not significant at the first immunization (Fig. 3). Thus, the data were consistent with the view that administration of txk expression plasmid induced T cells to produce IFN- γ , and as a result, Th1 cytokine vs. Th2 cytokine ratio of the mice administered txk plasmid was shifted.

Next, the spleen cells recovered from mice after secondary immunization (with administration of txk expression plasmid; Fig. 1) were similarly treated *ex vivo* and subsequent cytokine production *in vitro* was measured. We observed the up-regulation of the IFN- γ production by spleen cells from mice administered txk expression plasmid, not only immunizing antigen-specific stimulation such as OVA and Cry J1, but also Con A stimulation-enhanced IFN- γ production (Fig. 4). Again irrelevant antigen stimulation did not induce detectable levels of IFN- γ production (data not shown). It is known that cells in the cell cycle uptake exogenous DNA such as a plasmid, much better than the resting cells in G0 phase. Because the lymphocytes responding to the immunizing antigen (anti-OVA lymphocytes or anti-Cry J1 lymphocytes) are in the cell cycle, they may have taken up Txk plasmid better, than lymphocytes with other specificity that had not been activated by the immunizing antigen. Accordingly the lymphocytes responding to the antigen showed extensive proliferation and as a result, the lymphocytes having Txk

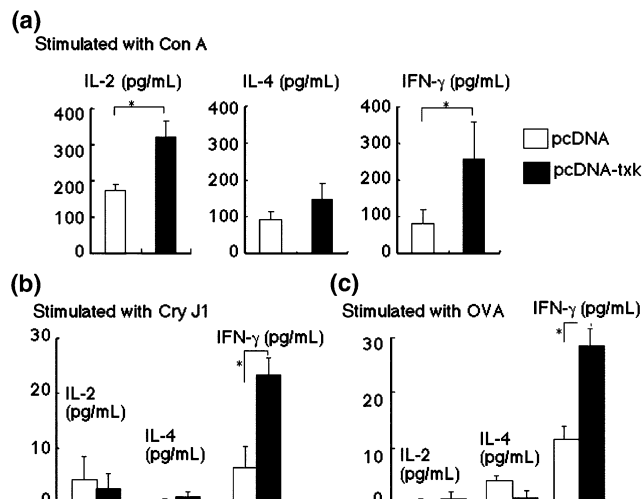


Fig. 4. *Ex vivo* cytokine production of spleen cells from mice given the txk expression plasmid: analysis after second immunization. (a and b) Effects of administration of txk expression plasmid on cytokine production by spleen cells after second immunization. First immunization with purified *Cryptomeria japonica* (Cry J1)+alum was conducted at day 0. Second immunization with the same antigen/adjuvant combination was conducted at day 21. At day 28, spleens of the 10 mice were recovered (please see Fig. 1). The spleen cells were stimulated with Concanavalin A (Con A) (a) and the immunizing antigen, Cry J1 (b) for 24 h *in vitro*. The 24 h-culture supernatants were recovered and assayed for the cytokine production. (c) In a separate series of experiments, the spleen cells from mice immunized with ovalbumin (OVA)+alum were tested for the cytokine production by OVA stimulation *ex vivo*. First and second immunization with OVA+alum were conducted at day 0 and day 21, respectively. At day 28, spleens of the 10 mice were recovered (please see Fig. 1). The spleen cells were stimulated with the immunizing antigen, OVA for 24 h *in vitro*. The 24 h-culture supernatants were recovered and assayed for the cytokine production. After consecutive administration of Txk plasmid, IFN- γ production was significantly enhanced by stimulation of both polyclonal activator (Con A) (a) and the immunizing antigen (Cry J1; b, and OVA; c). The irrelevant antigen, human γ -globulin (HGG) stimulation did not induce detectable levels of cytokine production. Thus the results were omitted. Results shown were mean+SD of 10 mice, and were representative of six independent experiments. * $P < 0.05$ by Mann-Whitney *U*-test.

plasmid may have become a major subpopulation in the spleen. Thus, the stimulation of Con A, a polyclonal activator, may have resulted in the up-regulation of IFN- γ production.

Finally, it should be noted that these *ex vivo* studies are likely to reflect *in vivo* IFN- γ production and subsequent Th1/Th2 shift. Thus it is possible that reflection of Th1/Th2 ratio *in vivo* may be altered in the mice given txk expression plasmid. To test this possibility, we have measured total IgG, IgG1, IgG2a, and IgE concentrations of the mice. We found that total IgG concentration in sera of the txk administered mice and that of control plasmid mice were comparable (Fig. 5). However, when we calculated IgG1/IgG2a ratio of the sera, the ratio was clearly reduced in the txk group, suggesting altered Th1/Th2 ratio in the mice. To our surprise, total IgE and OVA-specific IgE in sera were reduced in the txk group. Because we used OVA+alum for immunizing mice, it was reasonable to speculate that OVA-specific IgE constituted a majority of total IgE after the immunization. Thus, it is possible that specific reduction of OVA-specific IgE leads to the reduction of serum total IgE. These results suggest that txk administration induced Th1/Th2 shift leading to the reduction of IgE responses in the mice.

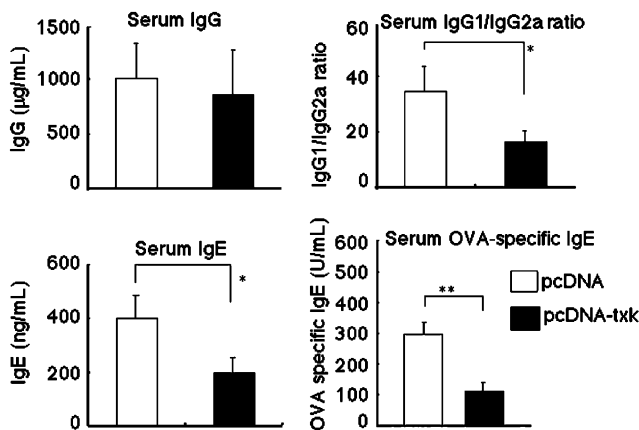


Fig. 5. Serum Ig concentration of mice given the txk expression plasmid. The normal BALB/c mice were immunized with OVA as shown in Fig. 1. After booster immunization with OVA + alum, sera were recovered, and total IgG, IgG1, IgG2a and IgE concentrations of the sera were determined individually by ELISA. OVA-specific IgE antibody in the serum of immunized mice was measured by IgE-capture ELISA. Results shown were mean + SD of each mouse group ($n = 5$). The same experiment was repeated four times. * $P < 0.05$ by Mann-Whitney U -test. ** $P < 0.05$ by Student's t -test.

Given the effect of administration of txk expression plasmid on IFN- γ secretion, it was logical to determine the effect of such plasmid administration on IL-12 secretion, because CpG motif of the txk expression plasmid may have induced IL-12 production. We thus determined serum levels of IL-12 of the mice given control plasmid and txk expression plasmid after 6, 24, and 48 h. The serum IL-12 levels were below the detection level in mice administered txk plasmid and those administered control plasmid (data not shown), suggesting that the plasmids did not provoke significant IL-12 production *in vivo* of the mice.

The development of an appropriate subset of Th cells is critical for determining the outcome of an immune response to various pathogens. Strongly polarized Th1/Th2 responses are manifested in chronic diseases such as autoimmune and allergic disorders and persistent infections [2–5, 36]. Thus, a means of altering Th phenotypes has immense importance for developing therapeutic strategies [37, 38].

Here, we demonstrate that intravenous administration of Txk plasmid renders Th cell cytokine production skewed towards Th1 dominant pattern. Our previous results suggest that differential distribution of Txk in Th1 and Th2 cells may be one of the main molecular events accounting for the distinction of the Th phenotype [30]. We have analysed surface phenotype of spleen cells from mice given txk expression plasmid. We found no significant differences of CD3 $^{+}$, CD4 $^{+}$, CD8 $^{+}$, CD25 $^{+}$, and B220 $^{+}$ cells in the spleens from mice given txk expression plasmid and those administered control plasmid (data not shown).

In this study, we explored the potential of intravenous delivery of plasmid encoding Txk with HVJ vector in antigen-specific immune responses in mice, and showed that the administered txk expression plasmid was highly effective for the shift to Th1 dominant responses.

For these observations to translate into therapy for human diseases, much more must be known about the persistence of the DNA in tissues and its possible harmful effects. However,

this is first demonstration for reduction of serum IgE concentrations without affecting serum IgG levels by administration of txk expression plasmid.

In conclusion, our study demonstrates that forced expression of Txk can bring about Th1 dominant immune responses. The potential of Txk has important implications for the treatment of autoimmune and allergic pathologies, which are often mediated by Th2 cells. The txk administration may, therefore, be applicable for therapeutic intervention in certain Th2-related diseases.

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References

- Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989; 7:145–73.
- Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature* 1996; 383:787–93.
- Romagnani S. Lymphokine production by human T cells in disease states. *Annu Rev Immunol* 1994; 12:227–57.
- Seder RA, Paul WE. Acquisition of lymphokine-producing phenotype by CD4 $^{+}$ T cells. *Annu Rev Immunol* 1994; 12:635–73.
- O'Garra A. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity* 1998; 8:275–83.
- Murphy KM, Ouyang W, Farrar JD et al. Signaling and transcription in T helper development. *Annu Rev Immunol* 2000; 18:451–94.
- Paul WE, Seder RA. Lymphocyte responses and cytokines. *Cell* 1994; 76:241–51.
- Asnagli H, Murphy KM. Stability and commitment in T helper cell development. *Curr Opin Immunol* 2001; 13:242–7.
- O'Garra A, Arai N. The molecular basis of T helper 1 and T helper 2 cell differentiation. *Trends Cell Biol* 2000; 10:542–50.
- Perez VL, Lederer JA, Lichtman AH, Abbas AK. Stability of Th1 and Th2 populations. *Int Immunol* 1995; 7:869–75.
- Szabo SJ, Dighe AS, Gubler U, Murphy KM. Regulation of the interleukin (IL)-12R beta 2 subunit expression in developing T helper 1 (Th1) and Th2 cells. *J Exp Med* 1997; 185:817–24.
- Rogge L, Barberis ML, Biffi M et al. Selective expression of an interleukin-12 receptor component by human T helper 1 cells. *J Exp Med* 1997; 185:825–31.
- Huang H, Paul WE. Impaired interleukin 4 signaling in T helper type 1 cells. *J Exp Med* 1998; 187:1305–13.
- Ho IC, Hodge MR, Rooney JW, Glimcher LH. The proto-oncogene c-maf is responsible for tissue-specific expression of interleukin-4. *Cell* 1996; 85:973–83.

- 15 Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 1997; 89:587–96.
- 16 Ouyang W, Jacobson NG, Bhattacharya D et al. The Ets transcription factor ERM is Th1-specific and induced by IL-12 through a Stat4-dependent pathway. *Proc Natl Acad Sci USA* 1999; 96:3888–93.
- 17 Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000; 100:655–69.
- 18 Arai N, Lee HJ, Ferber I, Kurata H, O'Garra A. Multiple levels of regulation of Th2 cytokine gene expression. *Cold Spring Harbor Symposia on Quantitative Biology Signaling & Gene Expression in the Immune System* 1999; 64:589–98.
- 19 Zhang DH, Cohn L, Ray P, Bottomly K, Ray A. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. *J Biol Chem* 1997; 272:21597–603.
- 20 Hu Q, Davidson D, Schwartzberg PL et al. Identification of Rlk, a novel protein tyrosine kinase with predominant expression in the T cell lineage. *J Biol Chem* 1995; 270:1928–34.
- 21 Sommers CL, Huang K, Shores EW et al. Murine txk: a protein tyrosine kinase gene regulated by T cell activation. *Oncogene* 1995; 11:245–51.
- 22 Sommers CL, Rabin RL, Grinberg A, Tsay HC, Farber J, Love PE. A role for the Tec family tyrosine kinase Txk in T cell activation and thymocyte selection. *J Exp Med* 1999; 190:1427–38.
- 23 Schaeffer EM, Debnath J, Yap G et al. Requirement for Tec kinases Rlk and Itk in T cell receptor signaling and immunity. *Science* 1999; 284:638–41.
- 24 Chamorro M, Czar MJ, Debnath J et al. Requirements for activation and RAFT localization of the T-lymphocyte kinase Rlk/Txk. *BMC Immunol* 2001; 2:3.
- 25 Czar MJ, Debnath J, Schaeffer EM, Lewis CM, Schwartzberg PL. Biochemical and genetic analyses of the Tec kinases Itk and Rlk/Txk. *Biochem Soc Trans* 2001; 29:863–7.
- 26 Schaeffer EM, Broussard C, Debnath J, Anderson S, McVicar DW, Schwartzberg PL. Tec family kinases modulate thresholds for thymocyte development and selection. *J Exp Med* 2000; 192: 987–1000.
- 27 Schneider H, Guerette B, Guntermann C, Rudd CE. Resting lymphocyte kinase (Rlk/Txk) targets lymphoid adaptor SLP-76 in the cooperative activation of interleukin-2 transcription in T-cells. *J Biol Chem* 2000; 275:3835–40.
- 28 Rajagopal K, Sommers CL, Decker DC et al. RIBP, a novel Rlk/Txk- and itk-binding adaptor protein that regulates T cell activation. *J Exp Med* 1999; 190:1657–68.
- 29 Takeba Y, Nagafuchi H, Takeno M, Kashiwakura J, Suzuki N. Txk, a member of non-receptor tyrosine kinase of Tec family, acts as a Th1 cell specific transcription factor and regulates IFN- γ gene transcription. *J Immunol* 2002; 168:2365–70.
- 30 Kashiwakura J, Suzuki N, Nagafuchi H et al. 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. *J Exp Med* 1999; 190:1147–54.
- 31 Kashiwakura J, Suzuki N, Takeno M et al. Evidence of autophosphorylation in Txk: Y91 is an autophosphorylation site. *Biol Pharm Bull* 2002; 25:718–21.
- 32 Kaneda Y, Nakajima T, Nishikawa T et al. Hemagglutinating virus of Japan (HVJ) envelope vector as a versatile gene delivery system. *Mol Ther* 2002; 6:219–26.
- 33 Sakaguchi M, Hashimoto M, Nigi H et al. Epitope specificity of IgE antibodies to a major allergen (Cry j 1) of Japanese cedar pollen in sera of humans and monkeys with pollinosis. *Immunology* 1997; 91:161–6.
- 34 Sakaguchi M, Inouye S, Miyazawa H, Tamura S. Measurement of antigen-specific mouse IgE by a fluorometric reverse (IgE-capture) ELISA. *J Immunol Methods* 1989; 116:181–7.
- 35 Miyagi T, Takeno M, Nagafuchi H, Takahashi M, Suzuki N. Flk1+ cells derived from mouse embryonic stem cells reconstitute hematopoiesis *in vivo* in SCID mice. *Exp Hematol* 2002; 30: 1444–53.
- 36 Liblau R, Singer S, McDevitt H. Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol Today* 1995; 16:34–8.
- 37 Finotto S, De Sanctis GT, Lehr HA et al. Treatment of allergic airway inflammation and hyperresponsiveness by antisense-induced local blockade of GATA-3 expression. *J Exp Med* 2001; 193:1247–60.
- 38 Neurath MF, Weigmann B, Finotto S et al. The transcription factor T-bet regulates mucosal T cell activation in experimental colitis and Crohn's disease. *J Exp Med* 2002; 195:1129–43.