

Constitutive expression of IRF-5 in HTLV-1-infected T cells

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Abstract. Human T-cell leukemia virus type 1 (HTLV-1) is the etiologic agent of adult T-cell leukemia (ATL), an aggressive and fatal leukemia of T cells. Interferon regulatory factor (IRF)-5 plays a critical role in the induction of interferon genes in viral infected cells. We examined the specific mechanisms underlying the expression and regulation of IRF-5 in HTLV-1-infected T cells. IRF-5 was constitutively transcribed into three distinct alternatively spliced isoforms (V1, V3 and V4) in HTLV-1-infected T-cell lines but not in uninfected T-cell lines. IRF-5 was also upregulated in HTLV-1-infected T-cell lines at protein level. Nuclear IRF-5 expression was noted in ATL cells present in lymph nodes and skin lesions. IRF-5 mRNA expression was induced following infection of T cells with HTLV-1, and specifically by viral oncoprotein Tax. Tax also activated V3 promoter. Microarray analysis of IRF-5-expressing uninfected T cells demonstrated that IRF-5 induced the expression of tumor necrosis factor family cytokines. The results suggest that IRF-5 is a Tax-regulated gene, and its expression may be associated with the pathogenesis of ATL.

Introduction

Interferon regulatory factor (IRF)-5 is a transcription factor member of the IRF family that regulates the expression of genes induced by viral infection (1). While IRF-5 was identified as a regulator of type I interferon (IFN) (2), further studies indicated that IRF-5 plays essential roles in the regulation of

genes involved in the stimulation of the immune system, cell growth, apoptosis and oncogenesis (3-6). Overexpression of IRF-5 has been associated with autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis (7,8). IRF-5 is a direct target of p53 (9) and displays some tumor suppressor properties as it can induce *p21*, *Bak*, *Bax* and *caspase-8* genes (6,10). Downregulation of IRF-5 by hypermethylation has been reported in hepatocellular carcinoma and gastric cancer (11,12). In contrast, IRF-5 is upregulated in thyroid cancer where it contributes to cell proliferation and survival (13). High-level expression of IRF-5 has been also detected in Hodgkin's lymphoma cells and considered to be crucial for their survival (14).

Human T-cell leukemia virus type 1 (HTLV-1) causes either adult T-cell leukemia (ATL) or chronic inflammatory disorders, such as HTLV-1-associated myelopathy/tropical spastic paraparesis, uveitis and arthritis (15,16). The HTLV-1 genome encodes the transactivator Tax protein that plays essential regulatory roles in HTLV-1 replication and oncogenic transformation of T lymphocytes (17,18). Tax modulates the activation of host signaling pathways to mediate cellular transformation and hyperstimulates the immune system (17,18). Therefore, viral Tax protein is considered to initiate the ATL-related leukemogenesis process, which subsequently progresses towards the ultimate leukemic stage through additional events occurring in Tax absence. In addition, Tax plays a central role in the pathophysiology of chronic inflammatory disorders (19).

With regard to the IRF family of transcription factors, several novel putative targets for Tax-mediated gene activation have been identified. IRF-3, a transcription factor critical in innate immunity to viral infection, is constitutively activated in a Tax-dependent manner (20). On the contrary, oncogenic IRF-4, another member in the IRF family of transcription factors, is overexpressed in lymphocytes of patients with ATL and HTLV-1-transformed T cells (21-25). Tax also induces the expression of IRF-4 (21,25-27). However, the expression of IRF-5 and related regulatory mechanisms have not been fully determined in HTLV-1-infected T cells. In this study, we determined the expression level of IRF-5 in HTLV-1-infected T cells. The results showed that IRF-5 expression was induced by Tax and that it regulated the expression of tumor necrosis factor (TNF) family cytokines.

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Materials and methods

Cell culture. The HTLV-1-infected MT-2, MT-4, C5/MJ, SLB-1, HUT-102, MT-1, TL-OmI and ED-40515(-) T-cell lines, and the negative control uninfected human leukemia Jurkat, MOLT-4 and CCRF-CEM T-cell lines, were grown in Roswell Park Memorial Institute-1640 medium supplemented with 10% heat-inactivated fetal bovine serum and antibiotics. JPX-9 cells are derivatives of Jurkat with *Tax* gene, which is stably integrated under the control of a metallothionein promoter (28). To induce *Tax* expression, JPX-9 cells were cultured in the presence of 20 μ M CdCl₂. The human acute monocytic leukemia cell line, THP-1, was set as a positive control for IRF-5 expression. TY8-3/MT-2 was established from the interleukin (IL)-2-dependent human T-cell line, TY8-3, co-cultured with mitomycin C (MMC)-treated MT-2 cells, and was capable of growth completely independent of IL-2 (29). Jurkat cells were stimulated with 1,000 U/ml of recombinant human IFN- α for the indicated time intervals.

HTLV-1 infection by co-cultivation. Peripheral blood mononuclear cells (PBMC) from a healthy donor were isolated from the heparinized blood sample by centrifugation over a Ficoll-Paque layer (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). MT-2 cells were pretreated with 200 μ g/ml of MMC for 60 min, pipetted vigorously, and washed three times with phosphate-buffered saline. PBMC and MMC-treated MT-2 cells were co-cultured in the presence of 10 ng/ml of IL-2. The culture medium was half-changed with fresh medium supplemented with IL-2 every 3 days. Since MT-2 cells were pretreated extensively with MMC, no discernible MT-2 cells were found.

RT-PCR. Total RNA was extracted from cells with TRIzol (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the protocol provided by the manufacturer. The RNA was reverse transcribed into cDNA using a PrimeScript™ RT-PCR kit (Takara Bio Inc., Otsu, Japan). The sequences of the primers for IRF-5, IFN- α , IFN- β , IFN- γ , 2',5'-oligoadenylate synthetase (2-5 AS), MxA, TNF- α , lymphotoxin (LT)- β , β -actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), *Tax* in HTLV-1-infected T cells, and *Tax* in JPX-9 cells are summarized in Table I (2,30-36).

Protein extraction and immunoblot analysis. Immunoblot analysis was performed on whole cell lysate, and nuclear and cytoplasmic fractions. Each protein was extracted as previously described (37-39), and subjected to sodium dodecyl sulfate-polyacrylamide gels and transferred to polyvinylidene difluoride membranes. The membranes were then probed for IRF-5 (Abnova Corp., Taipei, Taiwan), actin (NeoMarkers Inc., Fremont, CA, USA), lamin B (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) or Myc-Tag (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Mouse monoclonal antibody to *Tax*, Lt-4, was previously described (40). The bands were visualized using enhanced chemiluminescence kit (Amersham Biosciences Corp., Piscataway, NJ, USA).

Immunofluorescence assays. Cells were fixed in 4% paraformaldehyde and permeabilized with Triton X-100. Then, the cells were stained with mouse anti-IRF-5 antibody (Abnova

Corp.). For immunofluorescence studies, washed cells were incubated with anti-mouse secondary antibody conjugated with Alexa Fluor 488 (Invitrogen Life Technologies). The nuclei were stained with Hoechst 33342 (Wako Pure Chemical Industries, Ltd.). After final washing, the cells were examined under a Leica DMI6000 microscope (Leica Microsystems, Wetzlar, Germany). Mounted coverslips were viewed through a 63x oil immersion lens (NA1.4) on a Leica TCS confocal system.

Immunohistochemical analysis. The diagnosis of ATL was based on clinical features, hematological findings and the presence of anti-HTLV-1 antibodies in the serum. Biopsy samples were taken from the lesional skin and lymph nodes of four patients with ATL. IRF-5 immunohistochemistry was performed using an anti-IRF-5 antibody (Abnova Corp.) after pretreatment of the deparaffinized tissue sections with ready-to-use proteinase K (Dako, Carpinteria, CA, USA). The sections were counterstained with methyl green, hydrated in ethanol, cleaned in xylene, and mounted. The stained cells were examined under a light microscope (Axioskop 2 Plus) with an Achroplan 40x/0.65 lens (both from Zeiss, Jena, Germany). Images were acquired with an AxioCam MRC camera and AxioVision 4.7 software (Zeiss). A signed consent form was obtained from each tissue donor.

Plasmids. The IRF-5 P-V3 promoter fragment was cloned into the *Kpn*I and *Xho*I sites of pGL3-Basic vector (Promega Corp., Madison, WI, USA) (30). Details of the plasmid expressing the HTLV-1 *Tax* through β -actin promoter were published previously (41). The coding sequences of IRF-5 were cloned into the pcDNA4/myc-His A vector (Invitrogen Life Technologies).

Transfection and luciferase assay. Jurkat cells were transfected at 5×10^6 cells with the luciferase reporter plasmid, together with 0.5-5 μ g of the *Tax* expression vector by electroporation (250 V, 960 μ F). Each transfection included the pRL-TK plasmid (Promega Corp.) as an internal control for variation in transfection efficiency. After 48 h, transfected cells were collected by centrifugation, washed with phosphate-buffered saline, and lysed in reporter lysis buffer (Promega Corp.). The luciferase activity was determined by the Dual-Luciferase Reporter system (Promega Corp.) using the protocol supplied by the manufacturer.

Microarray analysis. Jurkat cells were transfected with a control pcDNA4/myc-His A vector or IRF-5 expression vector using MicroPorator MP-100 (Digital Bio Technology Co., Ltd., Seoul, Korea), pulsed three times at 1,325 V for 10 msec each. After 24 h, total RNA samples were prepared using the RNeasy Plus Mini kit (Qiagen, Hilden, Germany) and confirmed to be of good quality by Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Waldbronn, Germany). Microarray analysis using a SurePrint G3 Human GE 8x60 K Microarray kit version 2.0 (Agilent Technologies, Inc.) was performed as previously described (42).

Results

Expression of IRF-5 in HTLV-1-infected T-cell lines. To investigate IRF-5 expression in both HTLV-1-infected and

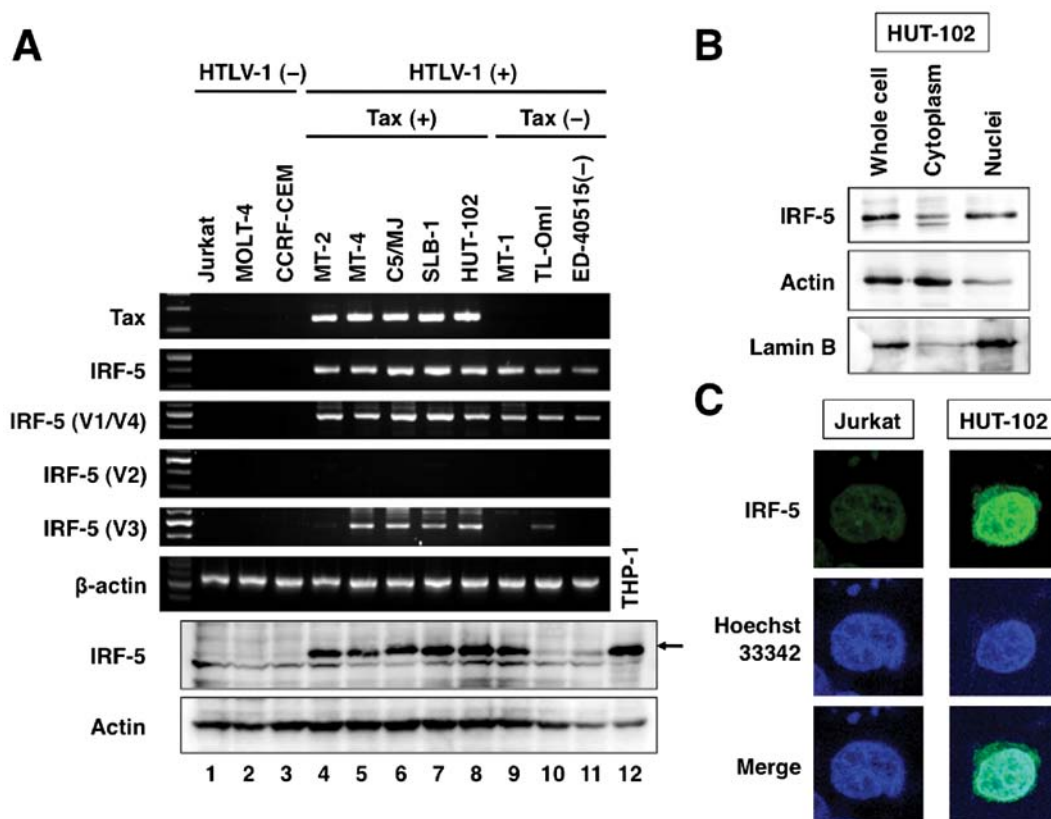


Figure 1. IRF-5 is expressed in HTLV-1-infected T-cell lines. (A) RT-PCR analysis of IRF-5 isoform expression and western blot analysis of IRF-5 expression in uninfected T-cell lines (lanes 1-3), HTLV-1-transformed T-cell lines (lanes 4-8) and ATL-derived T-cell lines (lanes 9-11). RT-PCR was performed with exon 1 isoform-specific primers that amplify through exon 4 (panels 3-5). Expression of β -actin was used as a control. Lysates from each sample were subjected to immunoblot using an anti-IRF-5 antibody (panel 7). Membranes were re-probed with the anti-actin antibody as a loading control (panel 8). THP-1 was used as positive control. (B) IRF-5 is localized primarily in the nuclei of HTLV-1-infected T cells. Whole cell lysate, and nuclear and cytoplasmic fractions were extracted and analyzed by immunoblotting for IRF-5. Actin and lamin B were used as quality controls to assess cytoplasmic and nuclear fraction purity and loading levels. (C) Jurkat and HUT-102 cells were subjected to immunofluorescence for IRF-5 using the secondary antibody conjugated with Alexa Fluor 488 (green). Hoechst 33342 (blue) was employed to identify the nuclei. The cells were visualized by confocal microscopy.

-uninfected T-cell lines, we collected three uninfected (Jurkat, MOLT-4 and CCRF-CEM), five HTLV-1-transformed (MT-2, MT-4, C5/MJ, SLB-1 and HUT-102) and three ATL-derived [MT-1, TL-Oml and ED-40515(-)] T-cell lines. All HTLV-1-transformed T-cell lines constitutively expressed Tax mRNA (Fig. 1A, panel 1). Expression of IRF-5 mRNA in 11 T-cell lines was analyzed by reverse-transcription polymerase chain reaction (RT-PCR) using IRF-5 specific primers. All eight of the HTLV-1-positive T-cell lines examined (lanes 4-11) strongly expressed IRF-5 mRNA, whereas the three HTLV-1-negative T-cell lines (lanes 1-3) did not (Fig. 1A, panel 2). IRF-5 is transcribed into various distinct alternatively spliced isoforms (30), and the IRF-5 variant 1 (V1), V2 and V3 transcripts have different noncoding first exons, whereas V1 and V4 share the same first exon. Primer sets that specifically recognize exon 1 of each isoform (Ex1V1, Ex1V2 and Ex1V3) and a common region in exon 4 of IRF-5 were optimized (Table I).

PCR amplification using the exon 1-specific sense primers was isoform-specific. We next examined the levels of constitutive exon 1-specific IRF-5 isoform expression in several T-cell lines (Fig. 1A, panels 3-5). Ex1V1 transcripts were detected in all HTLV-1-infected T-cell lines (Fig. 1A, panel 3). In comparison, transcripts associated with Ex1V2 could not be detected (Fig. 1A, panel 4). IRF-5 V3 transcript levels were specifically high in Tax-positive HTLV-1-transformed T-cell

lines (lanes 5-8), excluding MT-2, suggesting a possible role for Tax in enhanced IRF-5 Ex1V3 transcript levels (Fig. 1A, panel 5). High level of IRF-5 protein expression was evident in Tax-positive HTLV-1-transformed T-cell lines and ATL-derived MT-1 (Fig. 1A, panel 7, lanes 4-9). Intracellular mapping by immunoblotting and immunofluorescence staining showed high levels of IRF-5 in the nuclei of HTLV-1-infected HUT-102 cells (Fig. 1B and C). In contrast, almost no IRF-5 was detected in uninfected Jurkat cells (Fig. 1C).

Abundant IRF-5 expression in ATL cells in lymph nodes and skin lesions. Immunohistochemical staining of ATL cells in archived lymph nodes and skin tissue samples showed abundant IRF-5 in the nuclei of these cells (Fig. 2).

IRF-5 expression during HTLV-1 infection. To examine whether HTLV-1 infection induces IRF-5 expression, studies were performed using the parental TY8-3 cell line and TY8-3 cells infected with HTLV-1 (TY8-3/MT-2). TY8-3/MT-2 cells strongly expressed Tax mRNA (Fig. 3A). RT-PCR analysis also demonstrated upregulation of IRF-5 mRNA in TY8-3/MT-2 cells. To substantiate HTLV-1 control of IRF-5 expression in PBMC, we co-cultured PBMC and MMC-treated MT-2 cells. At seven days after co-cultivation, PBMC were harvested for assessment of expression of HTLV-1 viral gene by RT-PCR.

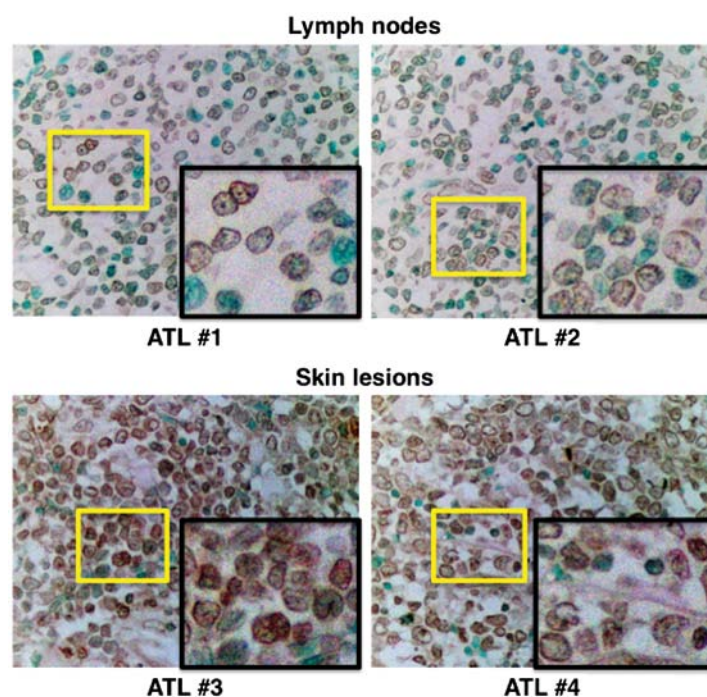


Figure 2. Representative results of immunohistochemical staining of IRF-5 in ATL lymph nodes and skin lesions. Tissue biopsy sections from ATL lymph nodes and skin lesions were stained with anti-IRF-5 antibody. Tissue sections were counterstained using methyl green. Original magnification, x400. Inset represents higher magnification of the small boxed region (original magnification, x1,000).

Table I. Primer sequences used in RT-PCR.

Name	Forward (5')	Reverse (3')
IRF-5	GCCTTGTTATTGCATGCCAGC	AGACCAAGCTTTTCAGCCTGG
IRF-5 (V1/4)	CCTGGCGCAGCCACGCAGGCGCA	CCAAAAGAGTAATCCTCAGGG
IRF-5 (V2)	GCGCCTGGAAAGCGAGCTCG	CCAAAAGAGTAATCCTCAGGG
IRF-5 (V3)	CTAGGCAGGTGCAACCCCAAAA	CCAAAAGAGTAATCCTCAGGG
IFN- α	CAGGAGGAGTTTGATGGCAACCAG	GACAACCTCCCAGGCACAAGGGC
IFN- β	ATGACCAACAAGTGTCTCCTCCAAA	GTTTCGGAGGTAACCTGTAAGTCTG
IFN- γ	ATGAAATATACAAGTTATATCTTGGCTTT	GATGCTCTTCGACCTCGAAACAGCAT
2-5 AS	CCAGGAAATTAGGAGACAGC	TGGCAGGGAGGAAGCAGGAG
MxA	GCATCCCAACCTCTATTACT	TGTCTTCAGTTCCTTTGTCC
TNF- α	ATGAGCACTGAAAGCATGATC	TCACAGGGCAATGATCCCAAAGTAGACCTGCCC
LT- β	AAGTGCCAGAGGAGGAGCC	TCCCGCTCGTCAGAAACGCC
Tax in HTLV-1-infected T cells	CCGGCGCTGCTCTCATCCCGGT	GGCCGAACATAGTCCCCCAGAG
Tax in JPX-9 cells	ATCGGCTCAGCTCTACAGTTCCT	ATTCGCTTGTAGGGAACATTGGT
β -actin	GTGGGGCGCCCCAGGCACCA	CTCCTTAATGTCACGCACGATTTC
GAPDH	GCCAAGGTCATCCATGACAACTTTGG	GCCTGCTTACCACCTTCTTGATGTC

PBMC co-cultured with MMC-treated MT-2 cells expressed Tax mRNA (Fig. 3B). Furthermore, IRF-5 expression levels increased in these cells following induction of HTLV-1 gene. Noteworthy, IRF-5 expression was still detected in PBMC at 14 days after co-cultivation, which expressed Tax at a very low level (Fig. 3B). These results indicate that HTLV-1 infection induces the expression of IRF-5 in PBMC as well as T-cell line.

Direct effect of Tax on expression of IRF-5. Tax gene product is the primary viral transactivator protein that modulates

the expression of both viral and cellular genes (17,18). To test whether Tax directly induces IRF-5 expression, we used JPX-9, the Jurkat subline carrying Tax under the control of the *metallothionein* gene promoter (28). This cell line has been widely used to examine the effect of Tax on the expression of various cellular genes (28). The results are shown in Fig. 3C. Treatment of JPX-9 with CdCl₂ rapidly induced Tax mRNA expression (panel 1). Similarly, Tax protein expression was induced within 24 h after addition of CdCl₂ and reached a maximal level within 120 h (panel 7). Expression of the *IRF-5*

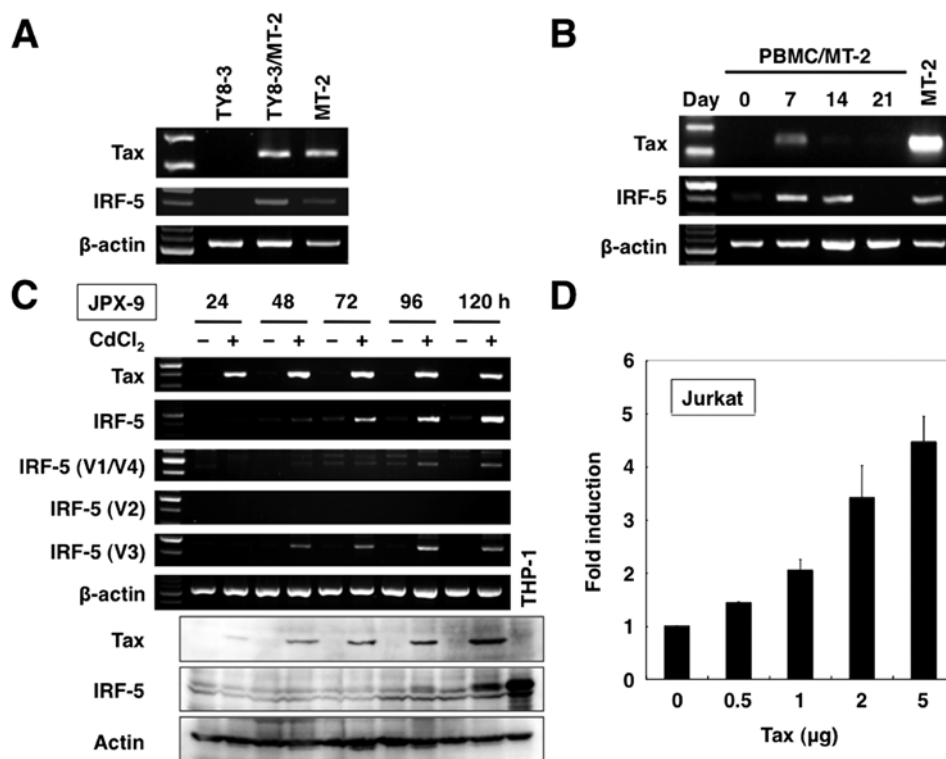


Figure 3. HTLV-1 infection and viral Tax expression induce IRF-5 expression. (A) RT-PCR analysis for mRNA expression of Tax and IRF-5 in TY8-3 and virally transformed TY8-3/MT-2 T cells. (B) Expression of Tax and IRF-5 in HTLV-1-infected PBMC. Normal PBMC were co-cultured with MMC-treated MT-2 cells. After co-cultivation, cells were harvested, and the expression of the indicated genes was analyzed by RT-PCR. (C) Induction of IRF-5 by Tax. JPX-9 cells were treated with 20 μM of CdCl₂ for 24-120 h. RT-PCR was carried out for the indicated genes (panels 1-6). Western blot analysis was also performed (panels 7-9). (D) Tax also regulates the P-V3 promoter. The P-V3 construct was co-transfected with increasing amounts of expression plasmid for Tax. In all transfection assays the levels of reporter firefly luciferase activity were normalized to a constant level of phRL-TK *Renilla* luciferase activity that served as an internal control. The results are presented as fold induction of luciferase activity of the P-V3 construct without Tax in Jurkat cells, which was considered 1. Data are mean ± SD of three independent experiments.

gene was not detected at 24 h but became evident within 48 h after addition of CdCl₂ (panel 2). A significant increase in the level of expression of IRF-5 protein (panel 8) was also noted; the protein level began to rise 96 h after the addition of CdCl₂ with the maximal level observed at 120 h.

We next examined IRF-5 isoform expression in CdCl₂-treated and -untreated JPX-9 cells. As shown in Fig. 3C, CdCl₂ upregulated Ex1 V1-associated transcript levels (panel 3), whereas V2 transcript levels were not detected in treated or untreated samples (panel 4). In comparison, CdCl₂ upregulated IRF-5 V3 transcript levels (panel 5).

We also examined the effects of Tax on IRF-5 isoform 3 promoter (P-V3). The activities of the reporter construct containing the entire P-V3 region were analyzed in transient transfection assays in Jurkat cells (Fig. 3D). Co-transfection of the P-V3 promoter construct in Jurkat cells with Tax expression plasmid enhanced promoter activity in Tax dose-dependent manner. Considered collectively, the above results indicate that Tax can activate IRF-5 P-V3 promoter.

Type I IFN does not regulate IRF-5. Type I IFN is a well-known transcriptional inducer of IRF-5 (10). However, its efficacy on IRF-5 induction in T cells is still unknown. Jurkat cells were exposed to IFN-α before RT-PCR. Unexpectedly, IFN-α failed to induce IRF-5 (Fig. 4A). However, treatment of these cells with IFN-α significantly increased the levels of other IFN-stimulated genes, *MxA* and *2-5 AS*.

IRF-5 targets TNF family cytokine genes. To assess the potential role of IRF-5 in initiating HTLV-1-infected cell-characteristic gene expression in T cells, Jurkat cells were transfected with control or IRF-5 expression plasmid. After 24 h of transfection, RT-PCR and immunoblotting were performed using specific primers, and anti-IRF-5 and anti-Myc antibodies to confirm transgene expression (Fig. 4B). In these experiments, changes in IRF-5-induced gene expression were analyzed by microarray analysis. The analysis showed significant enrichment of the up- and downregulated genes in IRF-5-transfected T cells. Table II shows genes that were upregulated by at least 5-fold in the presence of IRF-5. Previous studies reported that HTLV-1 infection induces T-cell activation and *in vitro* spontaneous lymphocyte proliferation, leading to the production of high levels of TNF-α in non-stimulated PBMC (43). Typical features of HTLV-1-infected T cells were recapitulated by IRF-5 activity, such as upregulation of TNF-α and LT-β (Table II and Fig. 4B).

Tax can also induce TNF-α expression. HTLV-1-infected T-cell lines constitutively secrete TNF-α (44). Furthermore, TNF-α expression is activated in the arthritic joints of Tax transgenic mice compared with the normal joints of non-transgenic mice (45). In the next set of experiments, we examined the effects of Tax on TNF-α expression in T cells. As expected, TNF-α was induced in Tax-expressing JPX-9 (Fig. 5A). Next, we used several T-cell lines to analyze the role of IRF-5 in the

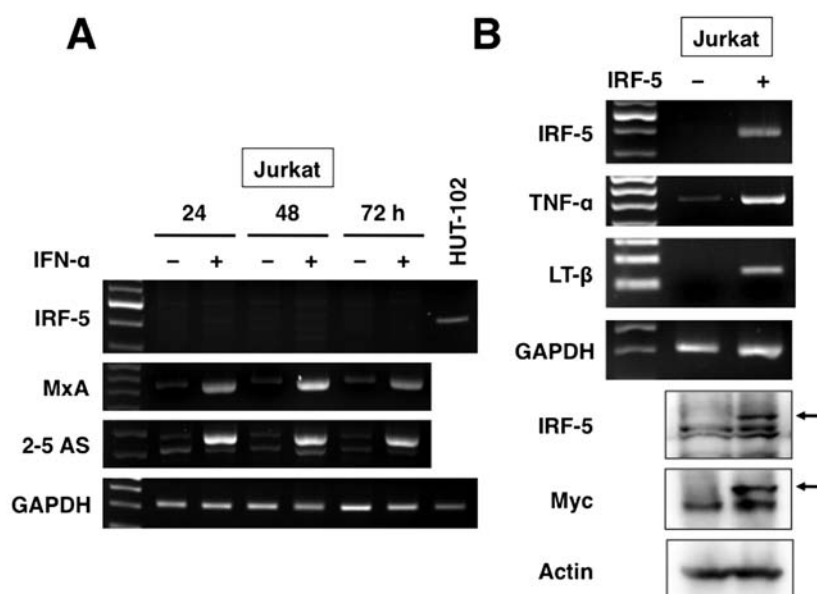


Figure 4. IFN- α does not induce IRF-5, and IRF-5 induces TNF family cytokines in T cells. (A) Jurkat cells were treated with IFN- α for the indicated time intervals, and subsequently analyzed by RT-PCR for the indicated IFN-regulatory genes. (B) IRF-5 induces the expression of TNF- α and LT- β in T cells. Jurkat cells were transfected with control or Myc-tagged IRF-5 expression plasmid. After 24 h, the expression levels of the indicated genes were determined by RT-PCR (panels 1-4). The level of Myc-tagged IRF-5 expression was also confirmed by immunoblotting with anti-IRF-5 (panel 5) and anti-Myc antibodies (panel 6). Left lane, empty vector control; right lane, Myc-tagged IRF-5 expressing Jurkat cells.

Table II. IRF-5-mediated genes with ≥ 5 fold change.

Symbol	Accession no.	Gene	Fold change
IL17F	NM_052872	Interleukin 17F	8.69
AZU1	NM_001700	Azurocidin 1	7.34
TNF	NM_000594	Tumor necrosis factor	7.24
PYY	NM_004160	Peptide YY	7.03
TEK	NM_000459	TEK tyrosine kinase, endothelial	6.82
LTB	NM_002341	Lymphotoxin β (TNF superfamily, member 3)	5.84
SLC24A4	NM_153646	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 4	5.76
ISPD	NM_001101426	Isoprenoid synthase domain containing	5.76
NRXN2	NM_138732	Neurexin 2	5.67
TMEM200A	NM_052913	Transmembrane protein 200A	5.65
SFRP1	NM_003012	Secreted frizzled-related protein 1	5.47
KIR2DS4	NM_012314	Killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 4	5.42
SDCBP2	NM_080489	Syndecan binding protein (syntenin) 2	5.42
KLHDC7B	NM_138433	Kelch domain containing 7B	5.38
FAM176A	NM_032181	Family with sequence similarity 176, member A	5.19
THADA	NM_001083953	Thyroid adenoma associated	5.18
GALNTL1	NM_020692	UDP-N-acetyl- α -D-galactosamine (polypeptide N-acetylgalactosaminyltransferase-like 1)	5.10
CGA	NM_000735	Glycoprotein hormones, α polypeptide	5.02
OR10A7	NM_001005280	Olfactory receptor, family 10, subfamily A, member 7	5.00

expression of type I and II IFN, and TNF- α . Compared to control T-cell lines, HTLV-1-infected T-cell lines expressed high levels of TNF- α (Fig. 5B). Similar to control T-cell lines, HTLV-1-infected T-cell lines also constitutively expressed IFN- α and - β . IFN- γ expression was not associated with HTLV-1 infection. There was no correlation between IFN and Tax expression in HTLV-1-infected T-cell lines. The

constitutive expression of TNF- α tended to be associated with IRF-5 or Tax expression.

Discussion

IRF-5 mRNA expression has been detected in B cells, dendritic cells, monocytes and natural killer cells but not in T cells (30).

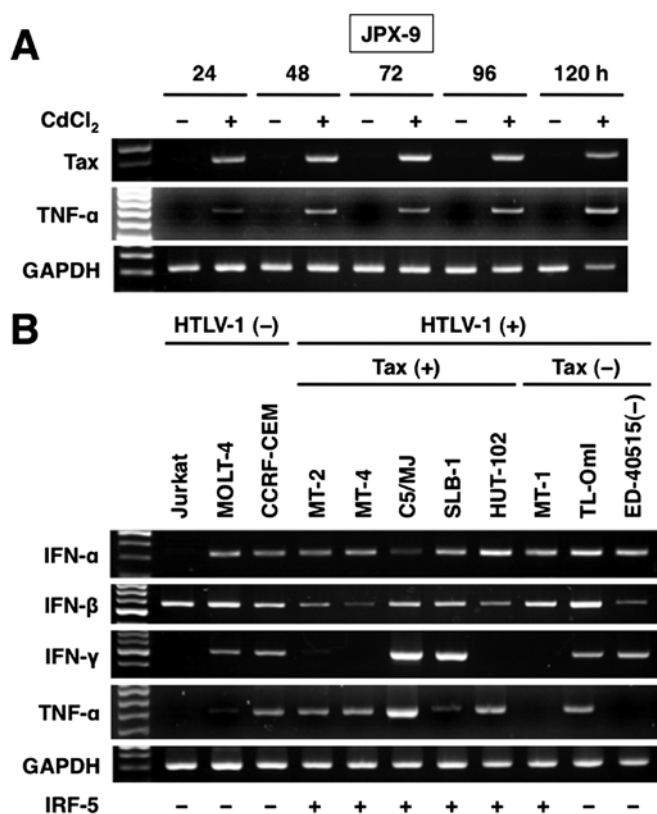


Figure 5. Induction of TNF-α by Tax, and expression of IFN and TNF-α in several T-cell lines. (A) Tax induces TNF-α expression. RT-PCR was carried out for TNF-α in JPX-9 cells treated with CdCl₂. (B) RT-PCR analysis for expression of IFN and TNF-α in the T-cell lines.

In the present study, we demonstrated that T cells acquire high levels of IRF-5 expression during HTLV-1 infection. Our results are consistent with those of oligonucleotide microarray reported by Baba *et al* (46), who identified IRF-5 to be one of the genes that were upregulated more than 40-fold in three HTLV-1-infected T-cell lines, including MT-2, compared with uninfected T-cell line MOLT-4. Our results also showed that the oncoprotein Tax activated the IRF-5 V3 promoter, and that IRF-5 was expressed in ATL cells infiltrating the lymph nodes and skin. Whether the expression of IRF-5 is present in PBMC samples from patients with ATL containing leukemic cells (which do not express Tax protein) was not examined and thus remains a very interesting question. Importantly, ATL-derived MT-1 cells, which also do not express Tax protein, expressed high levels of IRF-5 protein. These results suggest that Tax-independent IRF-5 expression mechanisms may also exist in ATL cells.

To establish the effects of endogenous IRF-5 in HTLV-1-infected T cells, we silenced its expression using the siRNA in HUT-102 cells (data not shown). Although IRF-5 silencing was confirmed by RT-PCR and western blot analysis, reduced IRF-5 expression did not affect cell growth (data not shown). In this study, the role of IRF-5 in T cells was investigated using IRF-5-expressing Jurkat cells and cDNA array technology. The results showed that IRF-5 expression does not affect the expression of cell growth- and apoptosis-related genes (data not shown). Furthermore, IRF-5 overexpression did not increase the proliferation of Jurkat cells (data not shown). These studies

confirmed that IRF-5 does not directly modulate cell growth. However, the exact IRF-5 function in cell proliferation needs to be further investigated using IRF-5 stable transfectants.

IRF-5 exists in multiple alternatively spliced isoforms that are expressed in a cell type-specific manner (30). The present results demonstrated that the induction of IRF-5 expression by Tax is isoform-specific. Tax-positive HTLV-1-infected T-cell lines specifically upregulated Ex1V3 transcripts, and the Ex1V3-specific transcripts were upregulated by Tax expression. Indeed, promoter reporter assays demonstrated that Tax enhanced P-V3 promoter activity.

IRF-5 is a central mediator that controls the expression of type I IFN (2). However, there was no link between IRF-5 and type I IFN expression in HTLV-1-infected T cell lines. In addition, IFN-α did not upregulate IRF-5 in Jurkat T cells. Although this finding does not completely exclude IFN-α-induced IRF-5 expression in primary peripheral T cells, the expression of type I IFN seems to be independent of IRF-5 in HTLV-1-infected T cells. On the contrary, IRF-5 expression induced other TNF family genes. TNF-α is a major cytokine involved in the promotion of inflammatory responses, and it plays a crucial role in the pathogenesis of various inflammatory, autoimmune and malignant diseases (47). Genetic polymorphisms leading to increased TNF-α production enhance susceptibility to not only ATL (48), but also to uveitis, another HTLV-1-related disease (49). TNF-α is suggested to contribute to the high levels of organ infiltration by leukemic cells in ATL (50). Tax can activate mouse TNF-α promoter through nuclear factor-κB (NF-κB) activation (51). Furthermore, IRF-5 can specifically interact with NF-κB RelA, and sustain TNF-α secretion in human dendritic cells (52). These findings suggest that Tax/NF-κB/IRF-5 may cooperate in HTLV-1-induced TNF-α promoter activation.

In summary, the present study indicates that high-level IRF-5 expression is specific to HTLV-1-infected T cells. The main function of IRF-5 in ATL and other HTLV-1-related disease should be investigated.

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