Transcriptional Activation of the Interleukin-21 Gene and Its Receptor Gene by Human T-cell Leukemia Virus Type 1 Tax in Human T-cells\textsuperscript{*}\textsuperscript{,#,1}

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At the incipient stages of the development of adult T-cell leukemia, T-cells infected with human T-cell leukemia virus type 1 (HTLV-1) suffer disregulation in cell growth caused by aberrant expression of host genes by the HTLV-1 transactivator protein Tax (Tax1). Tax1-mediated growth promotion is thought to result from, at least in part, up-regulation of genes for growth factors and their receptors that induce T-cell growth. In the present study, we demonstrate that Tax1 transactivates the interleukin-21 (IL-21) and its receptor (IL-21R) genes in human T-cells. Introduction of Tax1 via recombinant adenoviruses activated the promoters of the IL-21 and IL-21R genes. Isolated T-cells. We thus wondered whether Tax1 induces IL-21 and its receptor (IL-21R), because IL-21, which is produced by activated CD4\textsuperscript{+} T-cells, in contrast to Tax1. The study suggests insights into cytokine-dependent aberrant growth of HTLV-1-infected T-cells and the molecular basis of different pathogenicity between HTLV-1 and HTLV-2.

Human T-cell leukemia virus type 1 (HTLV-1)\textsuperscript{2} is an oncoretrovirus that is etiologically associated with adult T-cell leukemia (ATL)\textsuperscript{(1–3)} and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)\textsuperscript{(4, 5)}. Tax1 encoded by HTLV-1 is demonstrated to act as a key molecule in leukemogenesis through alteration of expression of cellular genes involved in cell proliferation and mortality\textsuperscript{(6)}. Molecular mechanisms of leukemogenesis by Tax1 have yet to be elucidated totally.

At the early stages of ATL development, HTLV-1-infected T-cells may induce their proliferation in two ways: Tax1 directly activates genes for cell growth such as cyclin D2, cdk4, and cdk6, leading to progression of the cell cycle\textsuperscript{(7–10)}, and, presumably simultaneously, cytokines and their receptors are induced by Tax1, which promotes cell growth in autocrine and/or paracrine manners. Inducible and transient expression of cytokines is crucial for proliferation and differentiation of lymphocytes. Early studies demonstrated that HTLV-1 infection promoted expression of various cytokines and their receptors, most notably the T-cell growth factor interleukin-2 (IL-2)\textsuperscript{(11–13)} and the subunit IL-2 receptor \(\alpha\) chain (IL-2R\(\alpha\)) of its high affinity receptor complex\textsuperscript{(14)}. Induction of the IL-2 and IL-2R\(\alpha\) genes in HTLV-1-infected cells is thought to be induced by Tax1 through activation of the NF-AT and NF-kB pathways, respectively\textsuperscript{(11–13)}. In addition to intrinsic cell cycle progression induced by Tax1, Tax1-mediated expression of IL-2 and IL-2R\(\alpha\) has been believed to contribute to ATL development by increased frequency of cell growth of infected cells.

HTLV-2 is closely related to HTLV-1 in genetic and biological terms, showing \(\sim 70\%\) sequence homology with each other\textsuperscript{(15)}. However, no link has been reported between infection with HTLV-2 and the development of ATL or another malignancies. HTLV-2 encodes Tax2, which shows \(\sim 75\%\) sequence homology to Tax1. Tax1 and Tax2 have been shown to play critical roles in immortalization of T-cells and maintenance of persistent infection with these viruses in human T-cells\textsuperscript{(16, 17)}. Recent studies demonstrate that Tax2 induces expression of IL-2, which promotes proliferation of HTLV-2-infected T-cell lines, but Tax1 does not transactivate the IL-2 gene\textsuperscript{(18)}. The findings prompted us to reconsider the notion that IL-2 autocrine loop supports cell proliferation of HTLV-1-infected T-cells. We thus wondered whether Tax1 induces IL-21 and its receptor (IL-21R), because IL-21, which is produced by activated CD4\textsuperscript{+} T-cells, effectively induces proliferation of T-cells in cooperation with other cytokines\textsuperscript{(19, 20)}. In addition to IL-2 and IL-15, which are close to IL-21 in terms of biological activ-
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ity and receptor constitution, IL-21 activates intracellular signaling pathways required for proliferation of T-cells through binding to its receptor, a complex consisting of two subunits, IL-21R and the common γ-chain (21). The common γ-chain is shared receptor complexes for IL-2, IL-4, IL-7, IL-9, and IL-15 besides IL-21. Among subunits of these receptor complexes, IL-2Rα, common γ-chain, and IL-15Rα are shown to be activated by Tax1 (14, 22–24).

In this study, we examined the effects of Tax1 and Tax2 on expression of the IL-21 and IL-21R genes and found that Tax1 induced the both genes in human T-cells. Moreover, our results indicated that Tax2 is distinct from Tax1 in effects on IL-21 gene expression in CD4+ T-cells. These observations imply that Tax1-mediated expression of the IL-21 and IL-21R genes may be involved in HTLV-1 pathogenesis.

EXPERIMENTAL PROCEDURES

Cells and Cell Culture—A human acute lymphocytic leukemia T-cell line Jurkat and its derivatives JPX-9 and JPX/M are maintained in RPMI 1640 medium containing 10% fetal calf serum. JPX-9 and JPX/M carry the Tax1 wild type and nonfunctional Tax1 mutant genes, respectively, under the control of the metallothionein promoter (25). Expression of Tax1 or its mutant was induced by the addition of 20 μM CdCl2. Peripheral blood lymphocytes (PBLs) were obtained from a consenting healthy adult by discontinuous density gradient sedimentation using Ficoll-Paque PLUS (GE Healthcare). CD4+ and CD8+ T-cells were separately enriched more than 92% from PBLs with CD4+ and CD8+ T Cell Isolation Kit II (Miltenyi Biotec), respectively. PBLs and CD4+ and CD8+ enriched populations were cultured in RPMI 1640 medium containing 20% fetal calf serum with 10 μg/ml phytohemagglutinin (PHA).

5′ Rapid Amplification of cDNA Ends (RACE)—5′ RACE was performed using the GeneRacer Kit (Invitrogen) according to the manufacturer’s protocol. Total RNA was isolated from PBLs treated with PHA for 72 h. The 5′ ends of cDNA were amplified by PCR with GeneRacer 5′ Primer (Invitrogen) and the following gene-specific primers: reverse IL-21 primer, 5′-agctggcagaaattcagggaccaag-3′; reverse IL-21R nested primer, 5′-gtggaccagtgtccccaagaagatg-3′; reverse IL-21 primers, 5′-tcatctgcttgcttgagatg-3′; and reverse IL-21R nested primer, 5′-ggagccacagacagccccctagggctc-3′. The PCR products were subcloned into pBlueScript II SK(−) vector (Stratagene), and the nucleotide sequences were determined.

Plasmid Construction—Expression vectors based on the human β-actin promoter for Tax1 (pMT-2Tax) and Tax2B (pHβAP-r-1-neoTax2B), whose original Tax2B clone was kindly provided by Dr. Hall, were described previously (26, 27). pHβAP-r-1-neo was used as a control plasmid (28). The fragment (−723 to +34) upstream of the IL-21 gene first exon was inserted in front of the luciferase gene in the reporter plasmid pGL3-basic (Promega), yielding pLl-21(−723)Luc. pLl-21R(−791)Luc was constructed by insertion of the 5′ regulatory region (−791 to +77) of the human IL-21 gene into the reporter plasmid pGL3-basic.

Isolation of RNA and Quantitative PCR—Total RNA was extracted using Isogen (Nippon Gene) according to the manufacturer’s protocol. First strand cDNA was synthesized using a first strand cDNA synthesis kit for reverse transcription-PCR (AMV; Roche Applied Science) according to the supplier’s protocol. Quantitative detection of mRNA for IL-2, IL-21, IL-21R, and Tax were performed by quantitative PCR with LightCycler (Roche Applied Science). Oligonucleotide primers used for IL-21R detection were as follows: forward, 5′-acatccctaggcccccTTgtgatg-3′; reverse, 5′-gagacagcagcagagacagggg-3′; and TaqMan probe, 5′-gggggtaggcagcagcgtgctg-TAMRA-3′. The primers and probe for IL-21 were described elsewhere (19), and those for IL-2 were purchased from Roche Applied Science. Expression levels of Tax1 and Tax2 mRNA were measured with a set of primers: forward, 5′-tctctcccacccagacac-gαac-3′; reverse, 5′-atggggggaagctgacttga-3′; and FastStart DNA Master SYBER Green (Roche Applied Science). The primers for 18 S rRNA were obtained from Takara. To generate standard curves for IL-21, IL-21R, and Tax mRNA, quantitative PCR was performed with serially diluted samples of plasmids, which carry the 511-bp IL-21 cDNA fragment (pEFneoHIL21) (29), the 124-bp (+1 to +124; see Fig. 5A) IL-21R cDNA fragment in pBluescript II, and the 1062-bp Tax1 fragment (pMT-2Tax), respectively. An IL-2 standard curve was similarly determined with commercially available products (Roche Applied Science). The copy numbers of IL-21, IL-21R, IL-2, and Tax mRNA were calculated based on respective standard curves and normalized against 18 S rRNA amounts, which were determined by quantitative PCR with the 123-base 18 S rRNA-derived single-stranded cDNA with the following primers: forward, 5′-actcaccagcagacctca-3′; and reverse, 5′-aacagacaataagctccac-3′.

Infection with Recombinant Adenoviruses—PBLs were stimulated with PHA for 72 h, washed with serum-free RPMI 1640, and cultured in RPMI 1640 containing 20% fetal calf serum for 48 h. The cells were infected with Tax1- and Tax2B-expressing recombinant adenoviruses or control virus (Ad-Con) at multiples of 100 plaque-forming units/cell as described (30, 31) and further cultured for 48 h. Recombinant adenoviruses for Tax1 (AxCAIY-Tax) and its mutant derivative Taxd17/5 (AxCAIY-Taxd17/5) were kindly provided by Dr. Yoshida (30). Another recombinant adenoviruses for Tax1 and Tax2B were newly generated using the ViraPower™ adenoviral expression system (Invitrogen) with the CAG promoter (kindly provided by Dr. Miyazaki) according to the supplier’s protocol, yielding Ad-Tax1 and Ad-Tax2B, respectively.

Immunoblotting Analysis—The cells were lysed in the radioimmune precipitation assay buffer containing 150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 1% Nonidet P-40, 0.5% deoxycholate, and 0.1% SDS. The cell extracts were separated by SDS-PAGE and blotted onto Immobilon-P membranes (Atto). The membranes were soaked in 5% skim milk solution and further incubated with antibodies specific for Tax1 (Lt-4) (32) or (Taxy-7) (33), Tax2 (GP3738) (34), and β-tubulin (sc-9104; Santa Cruz Biotechnology). The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies (GE Healthcare), and proteins were detected by the ECL plus Western blotting Detection System (GE Healthcare) according to the manufacturer’s protocol.

Immunostaining Analysis—For cell surface receptor staining, the cells were washed with phosphate-buffered saline containing 0.2% bovine serum albumin and stained with
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![Graphs A, B, C, D, E](https://example.com/graphs.png)

**A** IL-21/PBL
- IL-21/PBL
- IL-21/PBL
- IL-21/PBL
- IL-21/PBL

**B** IL-21R/PBL
- IL-21R/PBL
- IL-21R/PBL
- IL-21R/PBL
- IL-21R/PBL

**C** IL-21/Jurkat
- IL-21/Jurkat
- IL-21/Jurkat
- IL-21/Jurkat
- IL-21/Jurkat

**D** IL-21R/Jurkat
- IL-21R/Jurkat
- IL-21R/Jurkat
- IL-21R/Jurkat
- IL-21R/Jurkat

**E** Tax1
- Tax1
- Tax1
- Tax1
- Tax1

**FIGURE 1.** Tax1-mediated induction of IL-21 and IL-21R gene expression. PBLs (A and B) and Jurkat cells (C and D) were infected with a recombinant adenovirus expressing Tax1 (AxCAIY-Tax), its mutant derivative Taxd17/5 (AxCAIY-Taxd17/5), or control virus (Ad-Con). The cells were cultured for 48 h and harvested for RNA isolation. The levels of IL-21 (A and C) and IL-21R (B and D) mRNA were measured by quantitative PCR. IL-21 mRNA expression was under a detection level in Jurkat cells with None, Ad-Con, and Ad-Taxd17/5 infection. The results are shown as the means ± S.E. after normalization against 18S rRNA content. *, p < 0.05; E, expression of adenovirus-derived Tax1 and its mutant proteins in Jurkat cells was measured by immunoblotting with anti-Tax1 antibody (Taxy-7). The cell extracts were prepared 48 h after infection and separated by SDS-PAGE. The internal control is β-tubulin protein.

**RESULTS**

**Tax1 Induces IL-21 and IL-21R mRNA Expression**—Stimulation of normal human PBLs with phorbol myristate acetate and ionomycin has been reported to induce IL-21 and IL-21R gene expression, leading to growth of T-cells (19, 42). We examined whether expression of the IL-21 and IL-21R genes is induced by...
Tax1 in human T-cells. PBLs or Jurkat cells were infected with a recombinant adenovirus expressing Tax1 or its mutant derivative Taxd17/5 that lacks the transactivation ability, and the levels of IL-21 and IL-21R mRNA were measured by means of quantitative PCR. Tax1 significantly increased IL-21 mRNA expression in PBLs and Jurkat cells, whereas the Tax1 mutant was not effective in the elevation of IL-21 mRNA expression (Fig. 1, A and C). Expression of the IL-21R gene was also greatly enhanced upon introduction of Tax1, but not Taxd17/5, in Jurkat cells, which expressed no or little, if any, IL-21R mRNA in normal culture conditions (Fig. 1 D). Tax1 was also effective in an increase in IL-21R mRNA expression in PBLs, even though PBLs constitutively express IL-21R mRNA (Fig. 1B) (19). These results suggest that Tax1 potentially induces IL-21 and IL-21R gene expression in human T-cells.

**Tax1 Induces IL-21R Expression on the Cell Surface**—To assess the functional significance of Tax1-mediated IL-21R gene expression, cell surface expression of IL-21R was examined by flow cytometric analysis. Jurkat derivatives JPX-9 and JPX/M were used to take advantage of inducible expression of Tax1 and nonfunctional mutant Tax1, respectively. Upon metal ion-mediated induction of Tax1, IL-21R expression as well as IL-2Rα (14) was significantly induced 72 h post-stimulation in JPX-9 cells, whereas such induction was not seen in heavy metal-treated JPX/M cells (Fig. 2).

**The IL-21 Promoter Carries Two Tax1-responsive Elements**—To gain insights into molecular mechanisms of Tax1-induced IL-21 gene expression, the effects of Tax1 on an isolated IL-21 promoter were examined by means of reporter assays. Because the IL-21 transcriptional start site was not established yet, we determined the site (Fig. 3 A and supplemental Fig. S2). Jurkat cells were transiently transfected with the Tax1 expression plasmid (pMT-2Tax) along with a reporter plasmid containing the IL-21 wild type regulatory sequences (pIL-21Luc) or its 5′ end truncated mutants. Expression of Tax1 elevated the promoter activity of the IL-21 gene in this assay (Fig. 3B). Reporter assays with a series of 5′ end truncated promoters showed two-step decreases in Tax1-dependent activation of the IL-21 promoter (Fig. 3B). These results suggest that at least two regions, −492 to −329 (upstream) and −131 to −44 (downstream), in the IL-21 promoter may mediate activation of the promoter in response to Tax1. A computer search
NF-κB and AP-1 Bind to Tax1-responsive Elements in the IL-21 Promoter—Cellular factors that bind to Tax1-responsive elements were investigated by means of ChIP and gel mobility shift assays. Chromatin complexes cross-linked with nuclear factors were prepared from PBLs infected with Ad-Tax1 or Ad-Con and subjected to immunoprecipitation with antibodies against NF-κB and AP-1. Anti-p52/anti-RelB antibody mixture or anti-JunD antibody alone precipitated chromatin DNA fragments encompassing the IL-21 NF-κB and IL-21 AP-1 elements in a Tax1-dependent manner, whereas neither anti-p50/anti-c-Rel antibody combination, anti-Fra2 antibody, nor anti-p21 antibody gave appreciable PCR bands with the same primer combination (Fig. 4, A and C). No PCR band was amplified from anti-p52/anti-RelB or anti-JunD antibody-mediated immunoprecipitates with PCR primers for the β-actin gene promoter (Fig. 4, A and C). During ChIP experiments, we noted that infection with Ad-Con without the Tax1 gene induced a slight increase in binding of NF-κB or AP-1 (Fig. 4, A–D). Our results suggest that p52/RelB and JunD function as mediators in Tax1-dependent activation of the IL-21 promoter.

The notion was supported by gel mobility shift assays. The −439 to −398 fragment including the IL-21 NF-κB element formed complexes with the nuclear extract from Jurkat cells in a Tax1-dependent manner, which was inhibited by the addition of unlabeled wild type oligonucleotides but not mutant oligonucleotides (Fig. 4E). The IL-21 AP-1 element was similarly examined for factor recruitment by gel mobility shift assays. The probe (−112 to −85) generated complexes with the nuclear extract from Jurkat cells indicated that the upstream and downstream regions carry DNA sequence candidates of an NF-κB-binding site and an AP-1-binding site, respectively. To define the implications of these possible sites in Tax1-mediated activation, we generated IL-21 promoter derivatives with substutional mutations in each site or both sites (Fig. 3C). The introduction of mutation in each site reproducibly reduced IL-21 promoter activity by little, if any, effect on complex formation (Fig. 4F). These results suggest that p52/RelB and JunD function as mediators in Tax1-dependent activation of the IL-21 promoter.

The IL-21R Promoter Contains Two Tax1-responsive Elements—To understand the mechanism underlying Tax1-mediated transcriptional activation of the IL-21R gene, the IL-21R promoter was dissected for reporter assays. The transcriptional start site was assigned as the means ± S.E., *p < 0.05, **p < 0.01, ***p < 0.001.
that might bind nuclear factors IRF and AP-1, respectively. Both nuclear factors are shown to be activated by Tax1 (23, 39). The putative elements (IL-21R IRF and IL-21R AP-1) were examined for their transcriptional activity in response to Tax1 by introduction of mutations in the respective elements (Fig. 5C). Reporter plasmids with one mutated element reproducibly decreased promoter activities in response to Tax1. Disruption of the two elements exhibited significantly lower promoter activity than those with the wild type (Fig. 5D). These findings suggest that Tax1-dependent activation of the IL-21R promoter is mediated, at least partly, through the putative IRF and AP-1 elements.

**IRF and AP-1 Bind to the Tax1-responsive Elements in the IL-21R Promoter**—Transcription factors bound to the putative Tax1-responsive elements in the IL-21R promoter were explored by means of ChIP and gel mobility shift assays. In ChIP assay, cross-linked chromatin extracted from the Jurkat cells infected with Ad-Tax1 showed IL-21R promoter-derived specific PCR bands after immunoprecipitation with anti-IRF-1 or anti-JunD antibody (Fig. 6A–D). Anti-IRF-4 and anti-Fra2 antibodies were not effective in immunoprecipitation. These results were supported by observations shown by gel mobility shift assays. The probe encompassing the IL-21R IRF element formed complexes with the Jurkat nuclear extract in a Tax1-dependent manner (Fig. 6E). The complex formation was competed by the addition of either the IL-21R wild type oligonucleotides or typical IRF element derived from the low molecular mass polypeptide 2 (LMP2) gene promoter (Fig. 6E). IL-21R IRF element mutant oligonucleotides affected little, if any, complex formation with the IL-21R probe. When the IL-21R AP-1 element was used as a probe, the nuclear extract of Jurkat cells infected with Ad-Tax1 gave bands that were not seen with the nuclear extract from Ad-Con-infected Jurkat cells (Fig. 6F). The complex formation was disturbed by the addition of cognate oligonucleotides or the IL-8 promoter AP-1 element but not their mutants (Fig. 6F). These results illustrate that IRF-1 and
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FIGURE 5. Identification of Tax1-responsive elements in the IL-21R promoter. A, the transcriptional start site (+1) of the IL-21R gene was determined by 5′ RACE with total RNA isolated from PBLs treated with PHA for 72 h. B, reporter plasmids with a series of IL-21R promoter mutants were transfected into Jurkat cells together with the Tax1 expression plasmid (pMT-Tax1). Luciferase activity was determined 48 h post-transfection and normalized against protein content. The results are shown as the fold activation relative to luciferase activity of the IL-21R promoter. C, schematic view of the IL-21R promoter with lowercase letters indicating mutated nucleotides. D, Jurkat cells were transfected with a reporter plasmid carrying either the wild type promoter (pIL-21R(−791)Luc) or point mutated promoters (pIL-21R(−791) IRFmt)Luc, pIL-21R(−791) AP-1(m)Luc, and pIL-21R(−791) I(Amt)Luc) along with the Tax1 expression plasmid (pMT-Tax1), and the luciferase activities were determined. The results are shown as the means ± S.E. *, p < 0.05; **, p < 0.01.

Interestingly, Tax2 introduction did not show appreciable induction of IL-21 expression in CD4+ and CD8+ T-cell enriched subpopulations (Fig. 7, E and J, in contrast to the result with unfractonated PBLs (Fig. 7A). As expected, IL-2 mRNA expression was seen in CD4+ and CD8+ T-cell enriched populations, when they were infected with Ad-Tax2B, but not Ad-Tax1. These results were observed under conditions of parallel expression of Tax1 and Tax2 in respective cell populations (Fig. 7, C, D, G, H, K, and L) and imply that Tax1, but not Tax2, directly regulates transactivation of the IL-21 gene in a cell type-specific manner.

DISCUSSION

The present study demonstrates that Tax1 directly induces expression of IL-21 and IL-21R in human T-cells. Induction of IL-21 and IL-21R expression is mediated by activation of cellular transcription factors in response to Tax1; in the case of the IL-21 gene, NF-κB and AP-1 are found at least partly to be involved in activation, and IRF-1 and AP-1 participate in IL-21R gene activation. The pathways involving these transcription factors are shown to be activated by Tax1, direct association of Tax1 with NF-κB pathway molecules and JunD may be implicated in Tax1-dependent activation of the IL-21R promoter.

IL-21 Is Induced by Tax1 but Not Tax2—The effects of Tax1 on IL-21 expression were compared with those of Tax2 to elucidate differences in pathogenesis between HTLV-1 and HTLV-2. PBLs were infected with recombinant adenoviruses expressing Tax1 (Ad-Tax1) or Tax2 (Ad-Tax2B), and expression levels of mRNA for IL-21 and IL-22 were determined by means of quantitative PCR. Tax1 and Tax2 significantly elevated IL-21 gene expression in PBLs (Fig. 7A). Tax1 marginally activated expression of the IL-22 gene in PBLs, in contrast to Tax2, which profoundly induced expression of the same gene (Fig. 7B), as shown previously in Jurkat cells (18). Because previous studies reported that HTLV-2 caused preferential immortalization of CD8+ T-cells (15), we wished to examine the effects of Tax1 and Tax2 on T-cell subpopulations. CD4+ and CD8+ T-cells were enriched from PBLs and separately infected with the recombinant adenoviruses. IL-21 gene expression in the CD4+ T-cell enriched population was induced by Tax1, but Tax1 was not effective in expression of the IL-21 gene in CD8+ T-cell enriched population (Fig. 7, E and J). Tax1-mediated transcription of AP-1 family members, mainly JunD, is demonstrated (39, 43), and the IRF family is shown to interact with NF-κB and to be activated in HTLV-1-expressing T-cells (44, 45). The results with deletion mutants of the IL-21 and IL-21R promoters are somewhat different from those with their substitution mutants (Figs. 3 and 5), presumably suggesting that there would be other sites in both promoters that are involved in activation, and IRF-1 and AP-1 participate in IL-21R gene activation. The pathways involving these transcription factors are shown to be activated by Tax1; direct association of Tax1 with NF-κB pathway molecules and JunD may be implicated in Tax1-dependent activation of the IL-21R promoter.
element (Fig. 4A and data not shown). The specificity of NF-κB subunits is demonstrated to be dependent on nucleotide sequences of NF-κB-binding sites (47). The IL-21 expression in response to Tax1 is likely to utilize the noncanonical NF-κB complex (Fig. 4A). This observation is consistent with the results that a Tax1 mutant (Tax1ΔC), which lacks the C-terminal PDZ-binding motif that is necessary for efficient activation of the noncanonical NF-κB pathway, was significantly less effective in activation of the IL-21 gene promoter in reporter assay (supplemental Fig. S3F). Parallel expression of IL-21 and Tax1 was previously observed in HTLV-1-infected cell lines and ATL cell lines, although the authors did not indicate the correlation (48). In contrast to Tax1, Tax2B does not contain a PDZ-binding motif, and Tax2B is shown to lack the ability to activate the noncanonical pathway (49). Thus preferential IL-21 production in Tax1-producing but not Tax2-producing CD4+ T-cells (Fig. 7E) might reflect different activities of Tax1 and Tax2 to activate the NF-κB pathways.

Tax1 has been reported to transactivate the IL-2 gene (12, 50). The observation brought the assumption that aberrant growth of HTLV-1-infected T-cells in an IL-2-dependent manner expands an infected cell population, and among the population, some acquire IL-2-independent growth, finally leading to ATL development (6). Direct effects of Tax1 on induction of IL-2 gene transcription, however, seem unlikely (18). Rather, it may be possible to hypothesize that HTLV-1-infected cells pro-

![Figure 6](image-url)

**Figure 6.** Binding of IRF and AP-1 to Tax1-responsive elements in the IL-21R promoter. A–D, chromatin lysates from Jurkat cells infected with recombinant adenoviruses were sonicated and immunoprecipitated by anti-IRF-1 (A and B), anti-IRF-4 (A), anti-JunD (C and D), anti-Fra2 (C), and anti-p21 (A and C) antibodies. Purified DNA fragments were subjected to PCR with a set of primers specific to the IL-21R and β-actin promoters and followed by gel electrophoresis (A and C). The DNA fragments were subjected to quantitative PCR with same primer sets (B and D). The results are shown as the means ± S.E. after normalization on input DNA. *, p < 0.05. E and F, nuclear extracts from Jurkat cells infected with Ad-Tax1 (lanes 3 and 6–12, E, lanes 3 and 6–16, F) or Ad-Con (lanes 2 and 5) were incubated with the 30-bp oligonucleotides containing the IL-21R IRF element (E) or the 50-bp oligonucleotides containing the IL-21R AP-1 element (F). A typical IRF-binding site of the LMP2 promoter (E) and an AP-1-binding site of the IL-8 promoter (F) were used as controls. Competitors of unlabeled wild type and mutant oligonucleotides were added at 50 or 100 times molar excess prior to the addition of labeled probes. The arrows indicate possible specific complexes.
liferate in response to cytokines apart from IL-2, of which Tax1 positively influences their production. Our results suggest that IL-21 may be a possible candidate for induction of HTLV-1-infected cell growth in autocrine and paracrine manners. The notion does not exclude the possibility of IL-2 involvement in growth of HTLV-1-infected T-cells, because IL-2 may be released in immune responses to HTLV-1 infection. Like cooperation of IL-21 with IL-7 or IL-15, which delivers more effective growth signals in T-cells (19, 20), IL-21 in the presence of IL-2 may function as a stronger inducer for T-cell growth (20). Tax1 has also been reported to induce IL-15 mRNA (51).

IL-21 and IL-2 bind respective receptors on the cell surface, triggering growth signals into the nucleus through the cytoplasm. The two receptors are multi-subunit complexes containing the common γ chain (21), implying that two growth factors, IL-21 and IL-2, elicit common growth signal(s), besides respective signals association with unique subunits (IL-21R and IL-2Rβ) of the receptor complexes. IL-21 preferentially activates STAT1 and STAT3, whereas IL-2 and IL-15 primarily activate STAT5 (21, 52). STAT3 induces cell growth and survival of T-cells (53, 54). The transcription factors STAT3 and STAT5 are reported to be constitutively activated in HTLV-1-infected T-cells (43, 55). Our results demonstrate that Tax1 transcriptionally enhances IL-21R expression, generating functional IL-21R complex with the common γ chain. IL-21R is constitutively expressed in normal peripheral T-cells, and similarly, constitutive expression of the IL-21R complex is reported to be seen in HTLV-1-infected cell lines and ATL cells (48). The IL-21R complex may be internalized upon IL-21 binding. Tax1 could compensate decreases in surface expression of IL-21R by internalization, presumably resulting in maintaining functional IL-21R complexes at certain levels on the cell surface. This assumption is reminiscent of IL-2R complexes containing the common γ chain, which is also up-regulated by Tax1 (24). Tax1-mediated expression of IL-21 and its receptor may pro-
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dvide a mechanism that triggers aberrant proliferation of HTLV-1-infected T-cells prior to transformation.

Tax2B is demonstrated to induce the IL-2 gene via activation of the NF-AT pathway (18). In the present study, we show that Tax2 induces IL-21 expression in uninfected PBLs as high as Tax1 does, but an enriched normal CD4+ T-cell population is not a proper cell source for Tax2-mediated production of IL-21, and the same is the case with an enriched normal CD8+ T-cell population. These observations imply that Tax2-mediated production of IL-21 in PBLs might be a consequence of cytokine-cascade reactions in uninfected PBLs consisting of multiple blood cell lineages. It may be intriguing to speculate a link between pathogenesis and activity of Tax molecules of HTLV-1 and HTLV-2. Despite Tax2-dependent establishment of IL-2-dependent T-cell growth (18), there has been no clear demonstration that HTLV-2 infection is associated with any disease entity. This is quite a contrast to HTLV-1, which is closely associated with ATL and inflammatory diseases. The difference in pathogenesis between HTLV-1 and HTLV-2 may be, in part, attributable to the difference in Tax effects on the production of cytokines. Tax2 enhances production of IL-2 that supports T-cell growth in an autocrine manner, but it does not induce cytokine-independent immortalization of T-cells. On the other hand, Tax1 enhances IL-21 production, which might be associated with efficient conversion to cytokine-independent growth of HTLV-1-infected T-cells.

It may be noteworthy to learn the latest studies of implication of IL-21 in differentiation and amplification of Th17 cells (56, 57). Th17 cells are recently identified as a new subset of the effector T-cell family, an IL-17-producing T-cell population. Th17 cells are implicated in pathogenesis of various autoimmune conditions (58). The inflammatory disease HAM/TSP is caused by infection with HTLV-1, but among those infected with HTLV-2, a few sporadic cases of HAM/TSP are reported (15). Tax1-mediated expression of IL-21 and IL-21R might contribute to the onset of inflammatory diseases associated with HTLV-1 infection.

Acknowledgments—We thank M. Yoshida for recombinant adenoviruses, Y. Tanaka for anti-Tax1 antibodies and anti-IL-2Rα antibody, R. Mahieux for anti-Tax2 antibody, W. W. Hall for the Tax2B clone, J. Miyazaki for the expression vector pCAGGS, H. Nishitoh for technical assistance, and all of the members of our laboratory for various suggestions and encouragement.

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