RAPID COMMUNICATION

Transcriptional Regulation of the Human Interleukin-6 Gene Promoter in Human T-Cell Leukemia Virus Type I–Infected T-Cell Lines: Evidence for the Involvement of NF-κB

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Freshly isolated leukemic cells from patients with adult T-cell leukemia (ATL) and human T-cell leukemia virus type I (HTLV-I)–infected T-cell lines constitutively produce high levels of interleukin-6 (IL-6) protein and mRNA. To clarify the mechanisms that lead to the activation of IL-6 gene in HTLV-I–infected cells, we first studied the regulatory regions in the IL-6 gene transcription by transfection of chloramphenicol acetyltransferase (CAT) reporter plasmids containing the IL-6 promoter. When transfected into HTLV-I–infected T-cell lines MT-2 and HUT-102, IL-6 promoter/CAT plasmids were strongly activated without any stimulation. By deletion analysis of 5′ upstream region of IL-6 promoter, the DNA region between −73 and −59 bp from the transcription start site of IL-6 gene was important in the expression of IL-6/CAT activities in HTLV-I–infected cells. This region contains nuclear factor (NF)-κB binding site. The site-directed mutation of the κB motif in IL-6/CAT plasmid resulted in the complete abrogation of IL-6 promoter activity in these cells. Furthermore, when IL-6 promoter/CAT plasmid was introduced into an HTLV-I–uninfected T-cell line, Jurkat, IL-6 promoter activity was silent in the basal level, but strongly increased by the cotransfection with an HTLV-I–tax expression plasmid. However, tax expression plasmid showed no transactivation activity, when κB site was mutated in IL-6 promoter/CAT plasmid. We found that the IL-6 κB site specifically formed a complex with NF-κB–containing nuclear extracts from MT-2 and HUT-102 cells. Finally, transfection of HTLV-I tax into Jurkat cells resulted in induction of specific binding of nuclear extracts to the NF-κB sequence. These results strongly suggest that HTLV-I tax gene may transactivate IL-6 gene through κB site in HTLV-I–positive T-cell lines and activation of NF-κB may be crucial in HTLV-I–induced IL-6 gene activation in ATL.

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Human T-Cell Leukemia Virus Type I (HTLV-I) has been identified as the cause of the two disease states known as adult T-cell leukemia (ATL) and HTLV-I–associated myelopathy (HAM). These two diseases are characterized by distinct clinical and immunologic features: ATL is manifested as a lymphoproliferative disorder often with a concurrent compromised cellular immune state, and HAM is a hyperimmune state with an increased antibody titer to HTLV-I in serum and cerebrospinal fluid.

Interleukin-6 (IL-6) is a multifunctional cytokine that regulates central aspects of host defense responses. It is a major stimulus for the hepatic production of acute phase proteins. One of the major biologic functions of IL-6, which also led to its discovery as B-cell–stimulating factor 2, is its essential role in the maturation of activated B lymphocytes to antibody-producing cells.

Excessive production of IL-6 is associated with increased hepatic acute phase protein synthesis, thrombocytosis, and bone resorption. Many of these phenomena are also frequently observed in ATL. Previous studies have indicated that HTLV-I–transformed T-cell lines and HTLV-I–infected peripheral blood mononuclear cells (PBMCs) constitutively express IL-6. We recently reported that fresh leukemic cells from ATL patients constitutively express high levels of IL-6 mRNA and produce IL-6 protein. Thus, it is possible that IL-6 gene activation may contribute to these characteristic clinical features in ATL.

Originally, IL-6 was identified in fibroblasts, but diverse cell types are capable of producing IL-6. Mononuclear phagocytes, fibroblasts, and endothelial cells are major sources of IL-6. Interestingly, in contrast, IL-6 production by normal T or B cells appears to be rare. However, the purification and gene cloning of IL-6 were performed using HTLV-I–infected T-cell lines. Thus, the mechanism of IL-6 gene activation in HTLV-I–infected T-cell lines is important; however, none is known in the IL-6 gene regulation in these cells at present.

The tax protein encoded by the pX region of HTLV-I is capable of activating several cellular genes in vitro, in particular IL-2 and IL-2 receptor genes that are involved in T-cell activation. In the present report, we studied the mechanism of the IL-6 gene activation in HTLV-I–infected T-cell lines. Furthermore, we showed that HTLV-I tax is indeed able to activate the expression of the IL-6 promoter through NF-κB binding site and also induce the IL-6 κB binding activity in transfected Jurkat T cells. An understanding of the mechanism of IL-6 gene activation in HTLV-I–associated pathology will aid in design of agents to treat and prevent these diseases.

MATERIALS AND METHODS

Cells. All cell samples were derived from patients with ATL. All ATL patients had a high white blood cell count with typical
ATL lymphoblasts. Morphologic diagnosis was independently confirmed by immunophenotyping of isolated cells from the peripheral blood and by the demonstration by Southern blot analysis of monoclonal integration of HTLV-I genome (data not shown). Leukemic cells were isolated by standard procedures. Briefly, heparinized blood was layered on top of lymphocyte separation medium (Liton Bionetics, Kensington, MD) and mononuclear cells were recovered from the interface, washed with phosphate-buffered saline (PBS) and further incubated at 37°C for 2 hours in plastic culture dishes (Falcon 3003; Becton Dickinson, Oxnard, CA) to remove adherent cells. This solution was removed from the dishes and cells were washed thoroughly with PBS. ATL suspensions were then introduced into the 24-well culture plates (2 mL/well) at cell densities of 5 x 10^6/mL and cultured for 72 hours at 37°C in RPMI 1640 medium supplemented 10% fetal calf serum (FCS). Culture supernatants were collected by centrifugation to measure the levels of released IL-6. With one of the ATL patients (ATL 6), the PBMCs were further purified to obtain a highly enriched leukemic population. After isolation, more enriched ATL cell preparation was obtained by CD4-conjugated immunomagnetic beads (Dynal, Oslo, Norway). An aliquot of these cells was analyzed by fluorescence-activated cell sorting and found to be 98% CD4+ with <1% contamination with cells bearing the following markers: CD8, CD19, and CD14. MT-2 cells and HUT-102 cells (Fujisaki Cell Center, Okayama, Japan) are HTLV-I-infected human T-cell lines. An uninfected T-cell line, Jurkat, was used as a negative control. They were maintained in RPMI 1640 medium supplemented with 10% FCS at 37°C. Determination of IL-6 protein in culture supernatants. Cell-free supernatants were assessed for IL-6 protein by using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Toyo-Fuji Bionix Inc., Tokyo, Japan) according to manufacturer's instructions. The lower limit of assay is 10 pg/mL.

Oligonucleotide primers. The following primers were used for gene amplification: IL-6 sense, 5'-CTGAGAAGAGAGACAGTGAAC-AAGAGTAC; IL-6 antise, 5'-ACTGGTTCTGTGCGGTAC-GCT-TCCGAGC; tax sense, 5'-CCCACITCCCAGGGTITGGACAGA; tax antisense, 5'-CTGTAAGCTGACGGGATCAGGCG; P-actin sense, 5'-ACCAACTGGGAGCAGATGGAGA; and P-actin antisense, 5'-GTGGTGGTGAGTGAAGTCCG.

Reverse transcriptase polymerase chain reaction (RT-PCR). Total cellular RNA was isolated using the acid guanidinium thiocyanate-phenol-chloroform extraction method as previously described, and quantitation of the purified RNA was performed by absorbance at 260 nm. The integrity of the RNA was confirmed by electrophoresis under denaturing conditions on a 1% agarose gel. One microgram of RNA was used for cDNA synthesis at 42°C for 60 minutes with 6 U of Rous-associated virus 2 reverse transcriptase and 0.2 µg of DNA random hexadeoxynucleotide primers in a total volume of 20 µL. Then, half of cDNA reaction was amplified by 31 PCR cycles with 2.5 U of Taq polymerase in the presence of 1 µg each of the sense and antisense oligonucleotides. P-actin primers were used as an internal control. To assess integrity of RNA, PCR was performed in the same cDNA sample. Furthermore, the RT-PCR was performed with tax and P-actin primers. The thermal cycle profile was as follows: denaturation for 0.5 minutes at 94°C, annealing for 1 minute at 69°C, and extension for 1 minute at 72°C. Twenty microfilters of the PCR reaction mixture (100 µL total volume reaction) was subjected to electrophoresis in a 2% agarose visualized using ethidium bromide, and gave rise to bands of the sizes expected for the primers used (IL-6, 355 bp; tax, 203 bp; P-actin, 380 bp).

Plasmids. IL-6 promoter/chloramphenicol acetyltransferase (CAT) constructs, pIL-6CAT 512, pIL-6CAT 225, pIL-6CAT 138, pIL-6CAT 112, pIL-6CAT 73, and pIL-6CAT 58, and pH2R40M plasmid and pH2Rneo plasmid (kindly provided by Dr. M. Hatanaka, Kyoto University, Kyoto, Japan) have been previously described. Briefly, the plasmids pIL-6CAT 512, 225, 138, 112, 73, and 58 were constructed by insertion of six fragmental restriction sites from position -512, -225, -138, -112, -73, -58 bp to position +14 from the transcription start site, respectively, of the IL-6 gene into pBLCAT3 plasmid. Transcriptional induction of IL-6 gene by IL-1 was observed in all promoter constructs up to position -73 as previously described. The κB mutant (IL-6CAT ml) was prepared in the context of the pIL-6CAT 225 construct. The sequences of the NF-κB binding site are as follows: NF-κB, 5'-GGGATTITCCC-3'; mutant contains three base changes at the positions underlined: mt1, 5'-GGGATTITGGACAGA-3'. pH2Rneo (-tax) contains the simian virus 40 (SV40) sequences including the SV40 promoter and the SV40 polyadenylation signal, an R fragment of HTLV-I long-terminal repeat, and neomycin-resistant gene under control of the SV40 promoter. pH2R40M (-tax) has HindIII sites that can encode tax at an HindIII site of pH2Rneo.

DNA transfection. For DNA transfection, the diethyl aminoethyl (DEAE)-dextran method was used. MT-2 and HUT-102 cells were transfected with 10 µg of DNA per 1 x 10^6 cells. In Jurkat cells, 10 µg of pIL-6CAT 225 was either cotransfected with 10 µg of pH2R40M or pH2Rneo. Briefly, 1 x 10^6 cells were washed twice with serum-free RPMI 1640 medium and suspended in 10 mL of transfection cocktail, consisting of 10 µg of DNA, 50 mM/L TRIS-HCl (pH 7.2), and 2 mg of DEAE dextran (Pharmacia, Upsala, Sweden) in serum-free RPMI 1640 medium at room temperature. After 20 minutes, the cocktail was replaced by 10 mL of heparin (1.5 U/mL in RPMI 1640 medium; Sigma Chemical Co, St Louis, MO) to neutralize DEAE-dextran intoxication to the cells. After washing twice with 10 mL of RPMI 1640 medium, cells were suspended in RPMI 1640 supplemented 10% FCS, plated into dishes (Falcon 3003), cultured for 48 hours, harvested. All transfections were repeated at least three times with independent plasmid preparations.

CAT assay. After harvest, cells were washed twice with PBS and resuspended in 150 µL of 0.25 mol/L TRIS-HCl (pH 8.0). After three cycles of freezing and thawing, cell lysates were heat inactivated at 65°C for 10 minutes and microcentrifuged at 4°C for 30 minutes and protein concentrations in the supernatants were measured with the Bio-Rad protein assay kit (Bio-Rad, Richmond, CA). Reporter gene (CAT) activity was analyzed according to a liquid-phase acetylation method reported previously, using 100 µg of cell lysates in 150 µL and 100 µL of a mixture of 2.5 mM/L chloramphenicol (Sigma). Samples were counted on a Beckman liquid scintillation counter (Beckman Instruments, Irvine, CA) every 30 or 60 minutes continuously up to 16 hours. CAT activities were evaluated by calculating slopes within a linear range of the response. The activities of various CAT constructions were compared with that of pBLCAT3, which carries no promoter sequence upstream of the CAT gene, in unstimulated cells.

Preparation of nuclear extracts. Nuclear extracts were prepared by a method previously reported, with modifications. After harvest, 1 x 10^6 cells were washed with cold PBS, and incubated in 5 mL of buffer A (10 mM/L HEPES [pH 7.9] at 4°C, 5 mM/L MgCl2, 10 mM/L NaCl, 0.3 mol/L sucrose, 0.1 mol/L EGTA, 0.5 mM/L dithiothreitol [DTT], 0.5 mM/L phenylmethylsulfonyl fluoride [PMSF]) containing 1 µg of each of the protease inhibitors antipain, aprotinin, chymostatin, leupeptin, and pepstatin A per milliliter on ice for 15 minutes. After centrifugation, the cells were resuspended in 1 mL of buffer A with protease inhibitors and then Dounce homogenized (30 strokes). The homogenate was microcentrifuged for 0.5 minutes, and nuclei were resuspended in 0.4 mL of buffer B with protease inhibitors (20 mM/L HEPES [pH 7.9], 5 mM/L MgCl2, 0.3 mol/L
KCl, 0.2 mmol/L EGTA, 25% glycerol, 0.5 mmol/L EDTA, 0.5 mmol/L PMSF) and gently rocked on a platform at 4°C for 30 minutes. After 30 minutes of microcentrifugation at 4°C, supernatants were removed and dialyzed against 50 volumes of buffer D (20 mmol/L HEPES [pH 7.9], 0.1 mmol/L KCl, 0.2 mmol/L EDTA, 20% glycerol, 0.5 mmol/L DTT, 0.5 mmol/L PMSF) at 4°C overnight. After 30 minutes of microcentrifugation at 4°C, aliquots of supernatants were frozen at -80°C and protein concentrations were determined by using a Bio-Rad assay kit.

Electrophoretic mobility shift assays (EMSAs). EMSAs were performed essentially as previously described. Briefly, 5 μg of nuclear extracts was preincubated for 20 minutes at room temperature in 15 μL of a buffer (10 mmol/L TRIS-HCl [pH 7.5], 1 mmol/L EDTA, 1 mmol/L β-mercaptoethanol, 4% glycerol, 40 mmol/L NaCl) containing [32P]labeled probe (50 pg; ~20,000 cpm) and 50 ng of poly(dI-dC). The probe was an IL-6κB oligonucleotide containing a NF-κB binding site derived from the IL-6 promoter (~74 to ~62 from the transcription start site of the human gene; gatc-TGGGAGTTTTCCTCA). When indicated, 50 ng of unlabeled competitor DNA was added. E2F representing the P2-distal E2F-binding site of the c-myc promoter (~77 to ~65 from the P2 start site of the mouse gene; aagtcGCGTTCGCGGGAA) is a nonrelevant oligonucleotide; URE (aagccCGGTTTTCCTCAAAC) is an oligonucleotide containing an NF-κB–binding site derived from the murine c-myc promoter. The core sequence of NF-κB element is underlined. DNA-protein complexes were separated in a 4% polyacrylamide gel in 0.25 × TAE buffer (6.67 mmol/L TRIS-HCl [pH 7.5], 3.3 mmol/L sodium acetate, 1 mmol/L EDTA) and analyzed by autoradiography.

RESULTS

Expression of IL-6 and tax mRNA in ATL patients and HTLV-I–infected cell lines. We first studied the expression of the IL-6 gene in leukemic cells of ATL patients and HTLV-I–infected cell lines by RT-PCR method using the specific primers. A specific band with the expected size was visible as a signal in all samples from the 7 ATL patients and in two HTLV-I–infected cell lines (MT-2 and HUT-102) (Fig 1). Furthermore, similar results were obtained on fresh leukemic cells in the absence of any culture (data not shown). These PCR products were also confirmed by Southern analysis using IL-6 cDNA probe (data not shown). On the other hand, an HTLV-I–uninfected cell line (Jurkat) and control PBMCs did not show any detectable IL-6 transcript. In control experiments, the expression of β-actin mRNA was detectable in all samples, indicating the specific expression of the IL-6 gene in the ATL samples and in HTLV-I–infected cell lines.

RT-PCR was performed to examine the expression of tax mRNA in the same reaction as β-actin in these cells. The tax mRNA (203-bp band) was detected in all samples that were also positive for IL-6 mRNA (Fig 1). In contrast, Jurkat cell line and normal control PBMCs showed no tax expression. Thus, we conclude that in ATL patients, HTLV-I provirus is transcribed in ATL cells and tax mRNA is indeed expressed in vivo.

IL-6 secretion by HTLV-I–infected cell lines and fresh isolated ATL cells. As shown in Table 1, detectable levels of soluble IL-6 (ranging from 5,280 to 10,500 pg/mL) were present in the culture supernatants of HTLV-I–infected cell

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<th>Table 1. IL-6 Levels Assayed by ELISA</th>
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<td>Jurkat</td>
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<td>MT-2</td>
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<td>HUT-102</td>
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<td>Normal T cell</td>
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Cells were plated at 5 × 10⁶/mL and cultured for 72 hours in RPMI 1640 medium supplemented 10% FCS. Levels of IL-6 were measured by ELISA.
lines, MT-2 and HUT-102. In contrast, Jurkat T-cell line produced no detectable IL-6 protein even after phytohemagglutinin (PHA) or phorbol myristate acetate (PMA) activation (data not shown). In addition, supernatants from the unstimulated PBMCs derived from the ATL patients secreted high levels of IL-6 protein equivalent to those of HTLV-I-infected cell lines. To clarify a possibility that the increased IL-6 secretion from the ATL-derived PBMCs may result from the IL-6 production by a contamination of other cell types in the ATL cell preparation, we repurified a highly purified population of malignant ATL cells by magnetic beads and examined the level of IL-6 secretion in an ATL patient. The level of IL-6 secreted from the purified leukemia cells was 6,510 pg/mL and much higher. These results show that HTLV-I-infected cells secrete relatively large amounts of IL-6 protein. Therefore, we concentrated further study on the MT-2 and HUT-102 cell lines.

Analysis of the IL-6 promoter activity in HTLV-I-infected cells. To clarify whether excessive IL-6 production may be caused by the increase in transcription initiation, we next measured promoter activities by transfection of CAT reporter plasmids containing human IL-6 gene promoter. IL-6 promoter/CAT plasmid containing −512 to +14 bp upstream from the transcription start site of IL-6 gene (pIL-6CAT 512), which included full DNA region of IL-6 promoter activity, was transfected into MT-2 and HUT-102 cells. After 48 hours, cells were harvested and CAT activities were measured. As shown in Fig 2B, pIL-6CAT 512 showed threefold or fourfold increase in the level of CAT activity when compared with pBLCAT3, the reporter vector without a promoter insert, in MT-2 and HUT-102 cells. These results suggest that IL-6 promoter is activated in these cells. Next, to identify regions that are essential for the activation of the IL-6 promoter, we performed the 5′ site deletion analysis in CAT assays. As shown in Fig 2B, transcriptional activity was observed in all promoter constructs up to position −73, whereas the CAT activity dropped almost to the basal level when sequences between −73 and −59 were deleted (pIL-6CAT 58). The activities of these constructs in these cell lines indicated the presence of functional regulatory elements that allowed constitutive transcription to occur through the IL-6 promoter. This critical DNA region contains a sequence element (GGGATTTC argue −73 to −63) with a high homology with the NF-κB motif which was originally identified on the Igκ light-chain gene.23

To ascertain the relevance of this NF-κB binding site for the activation of the IL-6 promoter, we used a mutant IL-6 gene-CAT construct, in which the NF-κB binding site was mutated. Transcriptional induction of IL-6 gene by IL-1 was observed in all promoter constructs up to position −73 as previously described.19 On the other hand, the negative regulatory elements in IL-6 promoter activity may exist in the 5′-flanking region from −512 to −226 bp in HTLV-I-infected cell lines (Fig 2B). Therefore, this mutation, which converts three nucleotides within the NF-κB binding site (Fig 3A), was introduced into the pIL-6CAT 225 plasmid. Transfection of the mutated IL-6/CAT plasmid (IL-6CAT mt1) into MT-2 and HUT-102 cells showed that the activity of the IL-6 promoter (pIL-6CAT 225) was completely lost when compared with pBLCAT3, which was set at a value of 100. The bars represent the mean relative CAT activity calculated from three individual experiments. The designation of the different constructs is given at the left of the figure. The pBLCAT3 plasmid served as negative control.
Figure 4. Effect of mutated NF-κB element within context of the IL-6 gene on the transactivation by tax. Cotransfection of IL-6/CAT constructs with either tax expression plasmid (+tax) or control plasmid (−tax) into Jurkat cells. pBL-CAT3, pIL-6CAT 225 (wt), and IL-6CAT mt1, a mutated version of the pIL-6CAT 225 in which NF-κB binding site was mutated (mt1) are shown. Cytoplasmic extracts were obtained after 48 hours.

Induction of the binding of nuclear extracts to the NF-κB sequence by HTLV-I tax. The HTLV-I tax responses were then measured in Jurkat cells. Nuclear extracts were prepared from transiently transfected Jurkat cells with an expression plasmid for HTLV-I tax (pH2R40M) or a control plasmid (pH2Rneo), incubated with a labeled oligonucleotide probe corresponding to the IL-6κB site, and subjected to EMSAs. It was found that extracts prepared from pH2R40M-transfected Jurkat cells displayed induction of the complex (Fig 6). In contrast, the levels of complex remained unchanged after pH2Rneo transfection. NF-κB specificity was shown by elimination of complex after the addition of excess unlabeled IL-6κB or URE oligonucleotide, while no competition was obtained by an unrelated oligonucleotide, E2F. These results indicate that HTLV-I tax is indeed able to induce specific binding of nuclear extracts to the NF-κB sequence in the IL-6 gene promoter.

DISCUSSION

Although IL-6 is produced by diverse types of cells, IL-6 production by normal T and B cells appears to be rare. However, aberrant IL-6 production in several malignant cells of T- or B-cell lineages and its relationship to several diseases, including malignancy, have been reported. Although IL-6 has been purified and its gene has been cloned from HTLV-I-infected T-cell lines, the mechanism to regulate IL-6 production by HTLV-I-infected T-cell lines is not well understood.
late the aberrant IL-6 production in ATL has not been clarified at present. In this report, we confirmed the high levels of IL-6 protein and mRNA in HTLV-I-infected cells. In contrast, HTLV-I-uninfected Jurkat cells produce no IL-6 protein even after PHA or PMA activation.

The 5′ flanking region of the IL-6 gene contains several response elements that confer gene inducibility by the transcription factors AP-1 (−283 to −277), cyclic adenosine monophosphate−responding element-binding protein (−163 to −158), NF−IL-6 (−158 to −145), and NF−κB (−73 to −63). However, the functional significance of these or other potential protein−binding sites remains to be determined in HTLV-I−infected cells. We have analyzed the IL-6 promoter using deletion constructs of the 5′ region of the IL-6 gene linked to a CAT reporter gene. The activity of transfected IL-6 promoter constructs in MT-2 and HUT-102 cells correlates with their ability to constitutively produce IL-6. In MT-2 and HUT-102 cells, pIL-6CAT 225 was active in driving CAT gene transcription. pIL-6CAT 225 had approximately fivefold−greater activity than either pBLCAT3 or IL-6CAT mt1, but the level of IL-6 secreted from these cell lines was much higher than that of Jurkat cells. This discrepancy between promoter induction and the impressive IL-6 levels measured may be explained by an efficiency of transfection into these cell lines. High CAT activities were observed with constructs extending to position −73 of the IL-6 gene, and deletions progressing as far as position −59 completely abrogated IL-6 promoter activity. This critical region contains a κB consensus sequence (GGGATTTC) at positions −73 to −63. A mutation of this IL-6κB site completely abrogated the promoter activities in MT-2 and HUT-102 cells.

The HTLV-I tax gene activates the transcription of several cellular genes through the NF−κB transcription factor. The results of RT−PCR analysis showed that HTLV-I−infected cells express IL-6 also express tax mRNA. These informations suggest the possibility that tax transactivates the expression of IL-6. IL-6 promoter/CAT constructs showed no significant activity in an HTLV-I−uninfected cell line Jurkat. However, cotransfection of IL-6/CAT construct with an HTLV-I tax expression plasmid led to the detection of significant promoter activity in Jurkat cells. Furthermore, mutations within the NF−κB site of IL-6 gene abrogated the ability of tax to stimulate the transcriptional activity of the gene. The IL-6κB site competed with the c−myc κB sequence for binding to nuclear extracts from MT-2 and HUT-102 cells. Finally, transfection of tax into Jurkat cells resulted in induction of specific binding of nuclear extracts to the NF−κB sequence. Our results indicate that NF−κB site is an essential component in the induction of maximal production of IL-6, and that tax activation is at least one mechanism by which HTLV-I activates IL-6 production in T cells. However, it is known that in the peripheral blood cells of ATL patients, tax is not expressed or is expressed at a very low level. Thus, some other cellular functions or other viral genes such as Rex may also be involved in the impressive increase in IL-6 production in HTLV-I−transformed cells.

IL-6 is believed to play a key role in the development of multiple myeloma. It has been reported that an IL-6 autocrine proliferation of myeloma cells occurs via internal interaction between IL-6 and IL-6 receptor. The autocrine hypothesis suggests that one way in which a tumor may become autonomous is by production of growth factors for which it possesses functional receptors. However, HTLV-I−transformed cells, MT-2 and HUT-102 cells, appear not to be a candidate for an autocrine tumor because these cells display no receptor for IL-6 on their surface by flow cytofluorometry with the use of antihuman IL-6 receptor monoclonal antibody (data not shown).

The activation of IL-6 in HTLV-I−infected T cells suggests the participation of this cytokine in the pathogenesis of HTLV-I−induced diseases. Hypercalcemia (with and without osteolytic bone lesions), increase in acute−phase proteins, thrombocytosis, and fever are frequently observed in ATL patients. IL-6 stimulates bone resorption and acts synergistically with other osteoclast−activating factors in bone resorption. Our previous investigations showed that serum IL-
6 levels in ATL patients increased significantly, as compared with those of either asymptomatic carriers or controls. However, there was no correlation between IL-6 production levels and hypercalcemia. We are currently investigating the relationship between Ca level and IL-6 in the serum of ATL patients. On the other hand, it was shown that IL-6 can function as hepatocyte-stimulating factor to produce acute-phase proteins. It was also shown that IL-6 may induce fever as endogenous pyrogen. Therefore, several symptoms frequently observed in ATL patients could be explained by the presence of excessive IL-6 production in T cells.

Another manifestation of HTLV-I infection is HAM. It is of interest that Japanese HAM patients exhibit elevated levels of IL-6 in both serum and cerebrospinal fluid. It is conceivable that IL-6, which may cause B-cell activation, contributes to the elevation of serum Ig level in HAM patients.

Extensive clinical studies will be necessary in the future to define the in vivo significance of IL-6 activation by HTLV-I tax and its participation in disease processes during HTLV-I infection. However, this is the first report that clearly describes the transactivation mechanism of IL-6 gene by tax in T cells. Therefore, we believe that these findings may lead to a further understanding of the role of IL-6 in HTLV-I-associated pathogenesis. The next investigations to determine how the deregulation of this cytokine gene may play a role in HTLV-I-associated diseases will enable better design of drugs to prevent and treat HTLV-I-associated diseases.

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Transcriptional regulation of the human interleukin-6 gene promoter in human T-cell leukemia virus type I-infected T-cell lines: evidence for the involvement of NF-kappa B

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