Transactivation of the Transforming Growth Factor β1 (TGF-β1) Gene by Human T Lymphotropic Virus Type 1 Tax: A Potential Mechanism for the Increased Production of TGF-β1 in Adult T Cell Leukemia

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Summary

We examined the effect of the human T lymphotropic virus type 1 (HTLV-I) Tax gene product on the human transforming growth factor β1 (TGF-β1) promoter. Transfection of deleted constructs of the TGF-β1 promoter revealed regions homologous with AP-1 binding sites that were required for Tax-induced transactivation of the TGF-β1 promoter. In addition, we examined the expression and secretion of TGF-β in fresh leukemic cells isolated from patients with adult T cell leukemia (ATL) and in HTLV-1-infected T cell lines. We report that fresh leukemic cells from ATL patients constitutively produce high levels of TGF-β1 mRNA and secrete TGF-β1 but not TGF-β2 into the culture medium. In addition, long-term ATL cell lines expressed significant amounts of TGF-β1 mRNA as well as detectable levels of TGF-β1 protein. These results suggest a role for Tax in the upregulation of TGF-β1 in HTLV-I-infected cells.

A dult T cell leukemia (ATL)[1] is an aggressive, usually fatal T cell malignancy. Human T lymphotropic virus type I (HTLV-I) has been identified as the responsible infectious agent (1, 2). Clinical features include skin infiltration by the leukemic cells, hypercalcemia, and immunosuppression manifested by a high incidence of opportunistic infections (1, 3–9). Some of the clinical features of ATL have been ascribed to the overproduction of certain cytokines, such as TNF-α, IL-1, or lymphotoxin, by the tumor cells (10–13). Recently, excessive amounts of another cytokine, transforming growth factor β (TGF-β), have also been reported to be secreted by freshly isolated ATL cells (14–16).

The overproduction of TGF-β by these cells is intriguing since TGF-β has been shown to have potent immunosuppressive properties and to affect calcium metabolism in vitro (17–25). In vitro, TGF-β has been shown to depress the proliferative responses of thymocytes to IL-1, T cells to IL-2, and B cells to B cell growth factors. TGF-β also suppresses the generation of lymphokine-activated killer cells and cyto-

toxic T cells (19–25). There is evidence that TGF-β may be immunosuppressive in vivo as well. Patients with glioblastomas often have impaired cell-mediated immune responses, and their serum can inhibit mitogen- and antigen-induced proliferation of normal T cells (26); surgical removal of the tumor reversed the immunosuppression. Purification of the immune-suppressive factor from a glioblastoma cell line revealed it to be TGF-β2, a protein closely related to TGF-β1, with nearly identical biological properties. It has been postulated that TGF-β may also be involved in bone resorption since TGF-β activity is increased in cultures of fetal rat calvarial cells incubated with agents known to stimulate bone resorption, such as parathyroid hormone, vitamin D3, and IL-1 (17).

In this study, we have confirmed the overproduction of TGF-β by freshly isolated ATL cells (14) and have demonstrated that the TGF-β isoform secreted by these cells is exclusively TGF-β1. Moreover, since the TGF-β1 promoter was found to be transactivated by the HTLV-I p40^X (Tax) protein (27–29), we suggest that this may be a potential mechanism by which TGF-β production is increased in ATL cells. The specific sequence in the 5' regulatory region of the TGF-β promoter responsive to Tax was delineated by the use of deletion mutants and matched a consensus binding site for the transcriptional factor AP-1.

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[1] Abbreviations used in this paper: ATL, adult T cell leukemia; CAT, chloramphenicol acetyltransferase; HTLV-1, human T lymphotropic virus type 1; LTR, long terminal repeat; SELISA, sandwich ELISA; TGF, transforming growth factor; TPA, 12-O-tetradeconoyl-phorbil-13-acetate; TRE, TPA-responsive element.

Materials and Methods

Cells. Venous blood anticoagulated with either EGTA, heparin, or citrate was diluted 1:1 with PBS and then underlayered with lymphocyte separation medium (Organon Technika, Durham, NC). Tubes were spun at 600 g for 30 min at room temperature. Interface cells were washed three times in serum-free RPMI 1640 and suspended in one of two serum-free media preparations. The preparations used were AIMV (Gibco Laboratories, Grand Island, NY) and Nutridoma-HU–supplemented (1:100) RPMI 1640. Cultures were done in T-25 tissue culture flasks prepared in the following manner: 10 ml of PBS/25% FCS was added to each flask, and flasks were allowed to lie flat for 30 min at room temperature. This solution was removed from the flask and they were then washed thoroughly with PBS. ATL PBMC were then introduced into the flask at cell densities of 1.8–6 × 10^6/ml and cultured for 48–96 h at 37°C, 95% air/5% CO_2. Contents of the flask were removed and the cells were pelleted. The supernatants were spun again at 10,000 g for 15 min to remove residual cellular debris. With one of the ATL patients (ATL8), the PBMC were further purified to obtain a highly enriched leukemic population. After isolation, PBMC were depleted of monocytes by plastic adherence for 1 h at 37°C. The nonadherent lymphocytes were harvested and layered on 46.5% Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) and spun for 20 min at 600 g. The dense cells were harvested and washed. These dense cells were then rosetted with 2-aminoethylisothiouronium bromide (Sigma Chemical Co., St. Louis, MO)–treated SRBC at a final concentration of 1% for 20 min at 37°C. This suspension was underlayered with LSM and spun for 20 min at 600 g. The red cells in the pellet were lysed and the lymphocytes were collected. This lymphocyte population was suspended in a small volume, to which the antibodies OKT8 (anti-CD8) and 3A1 (anti-CD7) were added at 5 μg/ml in RPMI 1640/10% FCS. The suspension was incubated for 30 min at 4°C. Then, baby rabbit complement (Pel-Freez Biologicals, Rogers, AR) was added at 1:3 (vol/vol), and the suspension was incubated for 90 min at 37°C. The cell suspension was then underlayered with lymphocyte separation medium and spun at 800 g for 12 min. The interface cells were collected and put in culture with RPMI 1640/Nutridoma, as described above. An aliquot of these cells was analyzed by FACS and found to be >98.5% CD2+, CD4+, CD5+, with <1% contamination with cells bearing the following markers: CD7, CD8, CD19, CD14, and CD16. In addition, there was strong uniform expression of CD25 (Tac) on >88% of these cells. The presence of a large predominance of CD2+, CD4+, CD25+ cells with near absence of CD7+ or CD8+ cells indicates that there were few residual nonmalignant T cells in this population. Established HTLV-I-infected T cell lines MT-1, MT-2, and MT-4; T cell lines established from the peripheral blood of ATL patients, HUT-102; and HTLV-I-uninfected lines, Jurkat (T-cell line) and K-562 (human erythroleukemia), were cultured in RPMI 1640 medium with 10% (vol/vol) FCS. Human lung adenocarcinoma (A-549) cells were grown in DME with high glucose supplemented with 5% FCS.

Patient Population. Eight patients with a histologically confirmed diagnosis of ATL were studied. Each patient's diagnosis was established by the following criteria: (a) presence of a circulating pool of neoplastic mature T cells with indented nuclei; (b) expression of the α chain of the IL-2-R on >20% of the circulating lymphocytes; (c) demonstration of a clonal integration of the HTLV-I genome in the circulating lymphocytes, as well as a clonal rearrangement of the TCR β gene; and (d) presence of HTLV-I antibodies in the serum.

Analysis of TGF-β1 mRNA and TGF-β1-CAT mRNA Expression. PBMC from ATL patients were separated from heparinized venous blood by centrifugation over Ficoll-Hypaque. Cells were washed twice in RPMI 1640 and then lysed in a guanidinium thiocyanate solution. Total cellular RNA was isolated by the method of Chirgwin et al. (30). Total RNAs from resting T cells, PHA-activated T cells, purified normal PBMC, normal B cells, and *Staphylococcus aureus* Cowan–activated B cells were also isolated as described above. The levels of specific transcripts were analyzed by Northern blot analyses. Equal amounts of RNA (10 μg), as confirmed by ethidium staining, were subjected to electrophoresis on 1% agarose gels containing 0.66 M formaldehyde, and were transferred to nitrocellulose membranes. Blots were hybridized using [32P]-labeled probes according to the method of Church and Gilbert (31). Labeling of the 218-bp single-stranded TGF-β1 probe, complementary to the mature coding region of human TGF-β1 mRNA, has been described (32).

For the detection of chloramphenicol acetyltransferase (CAT) mRNA directed by TGF-β1 promoters, the levels of specific transcripts were analyzed by the RNase protection technique. RNA probes were synthesized according to the instructions of the manufacturer (Promega Biotec, Madison, WI). The EcoRI fragment from the phTG7 (33) was removed and religated. This plasmid contains a 256-bp CAT fragment. This plasmid was linearized by HindIII digestion, and the linear plasmid was transcribed with SP6 polymerase. DNase I was added to digest the DNA template. The 456-nucleotide RNA probe contained 256 bp of CAT coding sequence. Total RNA was harvested after transfection of TGF-β1 promoter CAT constructs, together with a second plasmid expressing the HTLV-I p40^X protein (34), and analyzed by RNase protection as described by Ondek et al. (35) using gel-purified probes specific for the CAT mRNA.

Plasmids, Transfections, and CAT Assays. TGF-β1-CAT constructs, phTG5, phTG H/B, phTG16, phTG17, phTG19, phTG22, phTG26, and phTG28 (33, 36, 37), and HTLV-I LTR-CAT and pHTLV-pX constructs (34) have been described in detail elsewhere. Transfections were performed by the $CaPO_4$ precipitation method (38) for A-549 cells and by the DEAE-dextran procedure, as described by Cross et al. (39), for Jurkat and K-562 cells. Approximately 10^7 cells of each type were transfected with 10 μg of plasmid DNA, together with 3 μg of the pHTLV-I-Tax plasmid. After 40 h, cells were harvested and extracts were assayed for CAT activity according to the method of Gorman et al. (40). In some experiments, transfected frequencies were monitored by cotransfection with 1 μg of pSVGH, a growth hormone expression vector.

Quantitation of the TGF-β1 and TGF-β2 Secreted by Cells in Culture. TGF-β secreted from the cells was quantitated by the sandwich ELISAs (SELISAs) for TGF-β1 and TGF-β2 using both turkey and rabbit neutralizing polyclonal antibodies against native TGF-βs (41).

Results

Effect of Tax on TGF-β1 Promoter Constructs and Identification of TGF-β1 Promoter Regions Required for Tax Transactivation. To understand the effect of the HTLV-I provirus on the TGF-β1 gene, we cotransfected plasmids containing sequences located 5' to the upstream transcriptional start site (the first promoter, phTG5), and located between the two major transcriptional initiation sites of the TGF-β1 gene (the second promoter) linked to the bacterial CAT gene (40) (Fig. 1 and see below) into A-549 cells, Jurkat cells, and K-562 cells, together with a HTLV-I Tax expression vector. As shown in Fig. 1, the first promoter CAT (phTG5) and the second promoter CAT (phTG16), as well as that of HTLV-I LTR CAT (34), were transactivated by Tax protein in all cells tested; expression
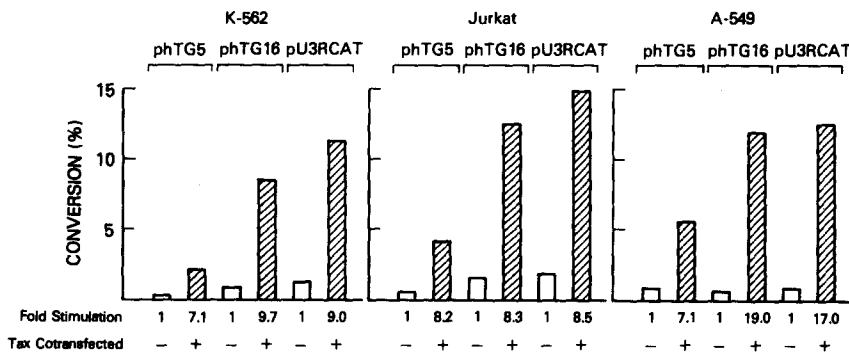
Figure 1. Activation of the plasmids phTG5, phTG16, and pHTLV-LTR-CAT (pU3RCAT) by Tax protein. Each of the plasmids was transfected into Jurkat cells, K-562 cells, and A-549 cells either with or without 3 μg of a p40^X-producing plasmid. 40 h later, the cells were harvested and CAT enzyme activity was determined. pHTLV-LTR-CAT (pU3RCAT) was used as a control for the Tax transactivation.

of phTG5 and pHTG16 is induced 7–19-fold in response to transactivation by Tax protein. These results suggest that common promoter elements required for Tax transactivation are present in the first and second promoters of the TGF-β1 gene. RNase protection analysis with an SP6 complementary RNA probe showed that the TGF-β1 CAT gene exhibited an increased level of RNA in cotransfection assays with the HTLV-I Tax expression vector, but not with pGEM4 (Fig. 2). It thus appears that transactivation of the TGF-β1 promoter by the Tax protein is at the transcriptional level.

To identify the specific sequences required for Tax transactivation, we used a series of 5' deletion mutants of the TGF-β1 promoters linked to the CAT gene. As shown in Fig. 3, the first promoter sequences of the TGF-β1 gene positioned between nucleotides −453 and −323 were required for optimal CAT induction by Tax protein. The second promoter of the TGF-β1 gene was also transactivated by Tax protein. The expression of plasmids phTG16, phTG17, phTG19, and phTG26 was increased by the Tax protein, whereas the Tax stimulation dropped almost to the basal level when sequences between +150 and +173 were deleted (phTG22). Tax stimulation again dropped substantially upon deletion of sequences between +247 and +267. These data suggest that the majority of activation by Tax protein is mediated by promoter sequences between positions −453 and −323 of the first promoter and between positions +150 and +173 and +247 and +267 in the second promoter of the TGF-β1 gene. These critical promoter sequences required for the Tax induction in TGF-β1 promoters contain sequence elements (TGTCTCA, −371 to −365; TGAGACGA, +160 to +167; and TGAGACT, +256 to +262) with a high degree of similarity to the AP-1 complex binding sites found in the collagenase (42) and metallothionein promoters (43). These TGF-β1 promoter elements play an important role in transcriptional activation of the TGF-β1 gene mediated by various inducers, including TGF-β1 (36, 37, 44) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (36, 45).

Expression of TGF-β1 mRNA in ATL Patients and HTLV-I-infected Cell Lines. To determine the levels of TGF-β1 gene expression in fresh leukemic cells isolated from patients with ATL and in HTLV-I-infected T cell lines, Northern blot analyses were performed with total RNA isolated from these leukemic cells. The 2.4-kb TGF-β1 mRNA was readily detected in six of six primary leukemic cells from patients with ATL
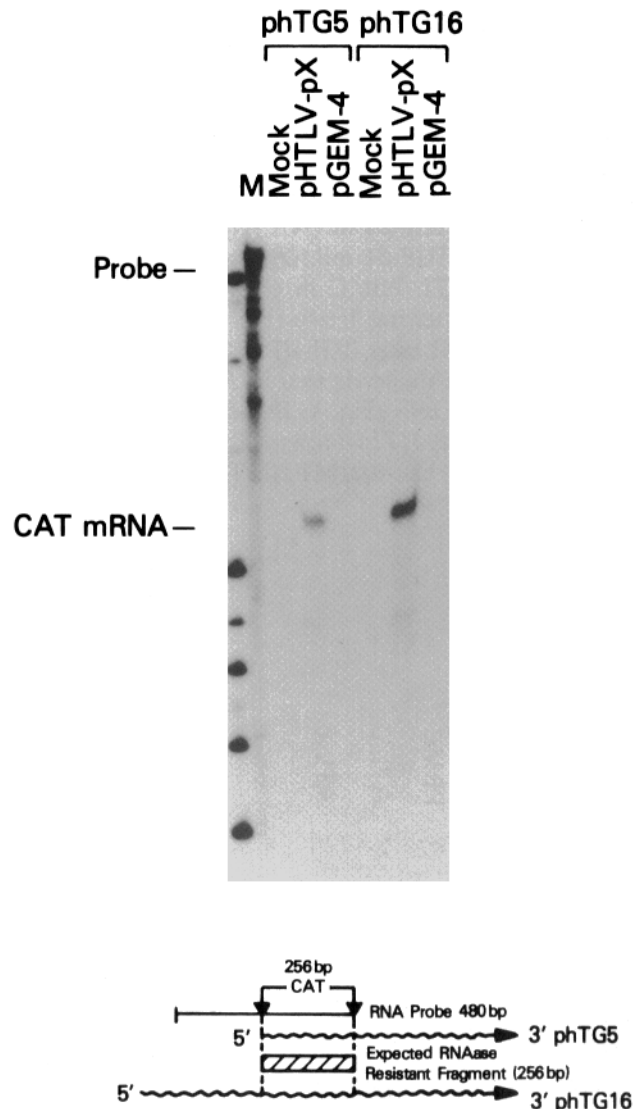

Figure 2. RNase protection analysis of TGF-β1-CAT gene expression. 10 μg of TGF-β1-CAT constructs, together with 3 μg of a p40^X-producing plasmid or 3 μg of the pGEM4 vector used as a control was transfected into A-549 cells. 40 h later, the total RNA was extracted and RNA was analyzed for the level of correctly initiated CAT transcript by RNase protection (35). The size of the CAT riboprobe is 480 nucleotides, whereas the size of the fragment protected from RNase digestion is 256 nucleotides.
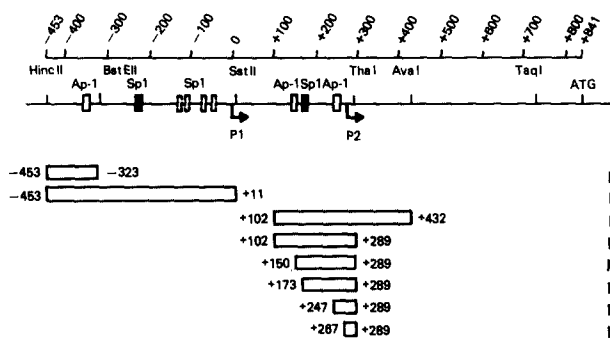
	RELATIVE CAT ACTIVITY	
	UNINDUCED	p40 tax
phTG H/B	1.19	7.38 (6.2)
phTG5	0.26	2.69 (7.7)
phTG16	0.87	8.40 (9.7)
phTG17	0.63	4.68 (7.4)
phTG19	0.65	4.75 (7.3)
phTG22	0.10	0.09 (0.9)
phTG26	0.21	1.70 (8.1)
phTG28	0.04	0.05 (1.3)

Figure 3. Tax stimulation of 5' deleted forms of human TGF-β1 promoters linked to the CAT reporter gene. On the top is an extended map of the two active promoter regions of the human TGF-β1 gene, indicating the two major transcription start sites (P1, P2), the locations of various protein-binding sites, and the positions of several restriction enzyme sites. Deletion mutants were transfected by the DEAE-dextran method (10 μg of DNA per 10^7 cells) into either Jurkat or K-562 cells, or by the calcium phosphate coprecipitation method into A-549 cells, either with or without 3 μg of p40^X-producing plasmid. CAT activity, expressed as the percentage of chloramphenicol transacetylation, was measured after 40 h, for a representative experiment using A549 cells, as described (40). Transfection frequencies were monitored by cotransfection with 1 μg of pSVGH, a growth hormone expression vector.

(Fig. 4, A and B), and in four of four HTLV-I-infected cell lines (Fig. 4 C). TGF-β1 mRNA was detected in the PHA/PMA-induced Jurkat RNA (positive control), whereas uninduced Jurkat cells, control resting T cells, and control PBMC had low levels of TGF-β1 mRNA (Fig. 4, A and C). Significantly higher TGF-β1 mRNA levels were observed in freshly isolated ATL PBMC in comparison with control PBMC or control resting T cells (Fig. 4 A). In RNA from five of six of the ATL cases, TGF-β1 mRNA levels were equal to or greater than the levels seen in ATL-derived cell lines or induced Jurkat cells (Fig. 4, B and C).

TGF-β Secretion by HTLV-I-infected T Cell Lines and Freshly Isolated ATL Cells. Hut-102, MT-1, MT-2, Jurkat, and normal

T and B cells were cultured at 37°C for 48 h in serum-free medium. The culture supernatant was harvested by centrifugation, and the amounts of TGF-β1 and TGF-β2 secreted from the cells were measured by SELISA. Supernatants conditioned by unactivated T cells or B cells contain low levels of TGF-β biological activity (<0.5 pM), while supernatants from PHA-activated T cells and SAC-activated B cells had levels 10–50-fold higher (Table 1) (20). However, as shown in Table 1, supernatants from the unstimulated PBMC derived from the ATL patients secreted levels of TGF-β equal to or substantially greater than those of the activated T and B cells. Since the increased TGF-β secretion from the ATL-derived PBMC could possibly result from the induction of
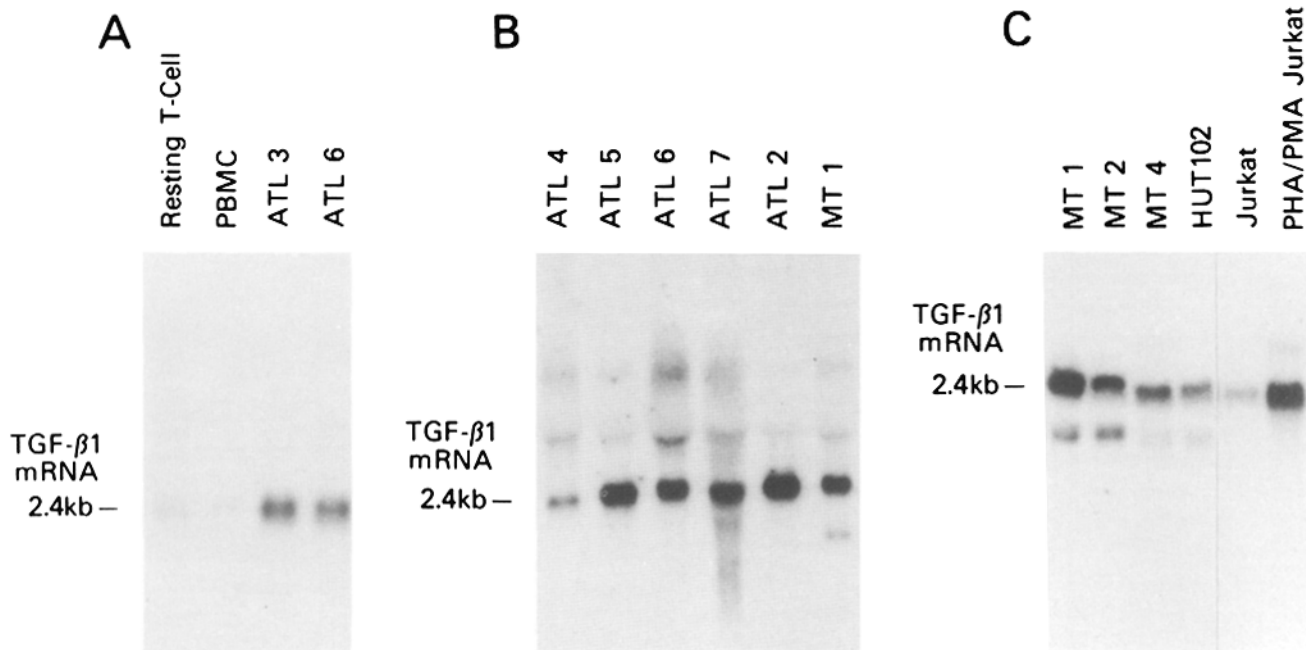

Figure 4. Detection of TGF-β1 mRNA in HTLV-I-infected cell lines and primary leukemic T cells. 10 μg RNA from control resting T cells (A); control PBMC (A); primary leukemic cells (ATL 2-7) (A and B); HTLV-1-infected T cell lines (MT1, MT2, MT4, and HUT-102) (C); and noninfected T cell line (Jurkat) (C) were hybridized to a radiolabeled TGF-β1 probe.

Table 1. *TGF-β Levels Assayed by SELISAs*

Cells	TGF-β1	TGF-β2
	pM	*pM*
Normal T cell	<0.50	<0.50
PHA-activated T cell	24.40 ± 0.4	<0.05
Normal B cell	<0.50	<0.05
SAC-activated B cell	7.92 ± 0.2	<0.50
Jurkat	<0.50	<0.50
MT1	4.43 ± 0.5	<0.50
MT2	6.92 ± 0.5	0.64 ± 0.1
HUT102	7.10 ± 0.4	<0.50
ATL1	80.40 ± 3.7	<0.50
ATL2	39.12 ± 2.3	1.09 ± 0.1
ATL3	8.91 ± 0.7	<0.50
ATL8	10.70 ± 0.2	<0.50

Cells were plated at 10^6/ml and cultured for 48 h in serum-free media as described in Materials and Methods. Levels of TGF-β1 and β2 peptides were measured by specific and sensitive SELISAs. Conditioned media TGF-β1 and TGF-β2 measured by the SELISAs were calculated using cubic and quadratic regression equations with Dynatech's immunosoft program. All values shown represent the average of the three determinations ± 1 SD from the mean.

TGF-β production in another cell type present in the PBMC preparation, we generated a highly purified population of malignant ATL cells and examined the level of TGF-β1 secreted (ATL8). The level of TGF-β1 secreted from the purified leukemic cells was much higher than that of unactivated T cells. The type of TGF-β secreted by HTLV-I-infected cells was determined by a SELISA using antisera specific for either TGF-β1 or TGF-β2 (41). TGF-β secreted from all of the tested cells was predominantly TGF-β1; levels of TGF-β2 were low to undetectable (Table 1). These results demonstrate that HTLV-I-infected cells secrete relatively large amounts of TGF-β1 protein.

Discussion

The present studies strongly suggest that Tax protein activates the gene for TGF-β1 through a distinct DNA element, the TPA-responsive element (TRE), which is also required for activation by TPA or TGF-β1. TRE motifs have been shown to bind the AP-1 complex consisting of the Jun/Fos dimer. In both the first and second promoters of the TGF-β1 gene, the DNA motifs that mediate the responses to Tax protein and to TGF-β1 autoinduction are identical.

The HTLV-I Tax protein (27–29) is a potent transcriptional activator of its own long terminal repeat (LTR) promoter and a number of cellular genes. It is known that the transactivation function of Tax is highly pleiotropic since Tax-responsive genes (e.g., IL-2-Rα [39, 46], IL-2 [46–48], GM-CSF [49], and IL-3 [50]) share little sequence homology in their regulatory sequences. We have documented that the essential Tax-responsive element within the HTLV-I LTR consists of a triply reiterated TGACGTCT motif (51, 52). In fact, it has been experimentally demonstrated that the HTLV-I LTR is both a cAMP and a phorbol ester responsive element. Recently, we have found that the TGACGTCT element for the HTLV-I LTR is a bona fide binding site for AP-1 and functionally responds to activation by v-jun (Jeang et al., manuscript in preparation). Our present observation that Tax also activates the TGF-β1 promoter through the AP-1/Jun motifs suggests that this described transactivation phenomenon may be physiologically relevant and may functionally occur through the signal transduction pathway that is postulated in Tax activation of the HTLV-I LTR (51, 52). Our results are also strengthened by the observation that expression of c-fos, which is one of the components of the AP-1 complex that binds to the TRE, is also transactivated by the Tax protein (53).

The mechanism for interaction of Tax protein with the AP-1 complex remains to be clarified. It is unlikely that Tax binds to DNA directly or leads to de novo synthesis of cellular factors that confer activation. One possible hypothesis might be that Tax expression results in the post-translational modification of a constitutively expressed transcription factor from an inactive to an active form, or that Tax participates directly in the formation of an active transcription complex. Thus, transactivation of TGF-β1 gene expression by Tax could proceed via an indirect, rather than direct, mechanism.

We also demonstrated high levels of constitutive expression of the TGF-β1 gene in leukemic cells from ATL patients, as well as in HTLV-I-infected T cell lines, confirming previous results (14). Since TGF-β mRNA levels from other leukemic cells, such as acute myelogeneous leukemia, acute lymphatic leukemia, and chronic myelogeneous leukemia, were low to undetectable (14), the fact that ATL cells produced high levels of TGF-β1 mRNA suggests that this event is directly induced by HTLV-I. In contrast to primary tumor cells, long-term ATL cell lines produced variable amounts of TGF-β1 mRNA.

The altered regulation by Tax of a variety of host genes, including those associated with T cell activation and proliferation, has led to speculation that Tax expression plays an essential role in the pathogenesis of HTLV-I-associated diseases. The high level of TGF-β1 gene expression associated with ATL cells suggests that this mediator may also play an important role in the pathological changes associated with this leukemia. However, efforts to date have failed to demonstrate Tax mRNA in fresh leukemic cells (54). Thus, expression of TGF-β1 in the circulating cells of ATL patients is similar to the IL-2-Rα (Tac), in that high levels of constitutive expression of both genes are easily demonstrated from fresh leukemic cells in the absence of Tax. Theoretically, transactivation of TGF-β1 and IL-2-Rα (Tac) could occur early in the course of HTLV-I infection, priming the T cells for a second "hit", resulting in leukemogenesis. Thus, the circulating ATL cells would be in a terminally differentiated, autonomous state and may no longer require Tax expression for proliferation. Alternatively, Tax expression may be limited to microenvironments in which cells are rapidly dividing, i.e., malignant lymph nodes. Finally, as a regulatory message, Tax mRNA may be present in minute quantities and may have a very short half-

life, rendering its detection extremely difficult. Using a sensitive PCR technique, we have occasionally detected Tax mRNA from ATL PBMC (unpublished observations, C.L. Tendler). Interestingly, Maeda et al. (55) have shown that many long-term cultured ATL cell lines no longer correspond to the original primary tumor cells based on the clonal pattern of rearrangement within the TCR β chain gene locus or in the integration site of the HTLV-1 provirus. Thus, it is clear that these two HTLV-I-infected T cell populations exhibit potentially important genetic and functional differences.

TGF-β is produced by mitogen-activated T and B cells, while nonactivated cells produce very low levels of TGF-β. In contrast, nonmitogen-activated HTLV-I-infected cell lines and freshly isolated leukemic cells from patients with ATL secreted levels of TGF-β equivalent to and/or in excess of mitogen-activated lymphocytes. Both normal lymphocytes and the HTLV-I-infected cells produced exclusively TGF-β1, with the exception of small amounts of TGF-β2 produced by MT2 cells and one of the patient's cells.

The TGF-β produced by normal lymphocytes is predominantly in a latent form that is unable to bind to the TGF-β receptor. This latent form is a complex between the remainder of the TGF-β precursor, mature TGF-β, and a third TGF-β binding protein (56, 57). In vitro, exposure to low pH or heat results in dissociation of the complex and the release of active TGF-β (56). The mechanism by which TGF-β is activated in vivo is poorly understood, but it may involve proteolytic enzymes or exposure to the appropriate microenvironment. Similar to normal lymphocytes, the TGF-β produced by the ATL cell lines appears to be predominantly latent. All the supernatants used in the SELISA were acid or heat inactivated before testing, which would activate any latent TGF-β present. However, the supernatants were also tested using a CCL-64 assay (58), a biologic assay that measures active TGF-β. Active TGF-β was detected in the supernatants conditioned by the ATL cells only after heat activation (A. B. Roberts, unpublished observation). This is in contrast to the findings of Niitsu et al. (14), who reported that much of the TGF-β produced by ATL cells was active. The distinction between the production of predominantly latent vs. active TGF-β is important. The half-life of active TGF-β in the circulation is exceedingly short ($\sim$3 min) (59), while the half-life of latent TGF-β is nearly 2 h (L. Wakefield, personal communication). Thus, the production of active TGF-β by a cell is likely to have an effect only in the immediate environment of the secreting cells, while the production of latent TGF-β by a cell may result in a more long-range effect.

Cellular and humoral immune responses are markedly impaired in patients with ATL. Abnormalities include disordered helper and suppressor T cell function, impaired mitogen responsiveness, decreased killer cell induction, and depressed B cell Ig synthesis (60–62). Additionally, immunosuppressive factors have been detected in HTLV-I-infected ATL sera and ATL culture supernatants (63, 64). X-irradiated leukemic cells from ATL patients suppress the PWM-induced Ig synthesis by control PBMC (65). The excessive production of TGF-β by ATL cells in vivo could account for many of these immunological abnormalities. TGF-β has been shown in vitro to impair both T and B cell proliferation, to inhibit NK cell induction and cytotoxic T cell induction, and to depress B cell Ig secretion (19–25). The in vitro effects of TGF-β and the observed immunosuppression in ATL patients are strikingly similar. Additionally, the excessive production of TGF-β by ATL cells may offer a selective advantage. Increased levels of TGF-β may suppress the very immune cells responsible for the elimination of the tumor cells.

TGF-βs are important in the regulation of many other cell types as well. Of interest to this study, they have been found to be important in the processes of bone resorption and new bone formation (17, 18). One of the important clinical features of ATL patients is the frequent hypercalcemia (1, 3–9). The findings that bone resorbing factors are present in the in vitro culture supernatants of ATL cells has suggested that osteoclast-activating factors are produced by the ATL cells themselves. Several factors, including IL-1β and TNF-β, have been detected in these supernatants and have been suggested to be involved in the pathogenesis of the hypercalcemia (12, 13). It is likely that TGF-β is involved as well, either by itself or in combination with these other cytokines. To establish firmly a relationship between TGF-β production and ATL-associated changes in calcium metabolism and immune competence, prospective studies correlating TGF-β production with changes in bone turnover, osteoclast activation, and immune function are needed.

In conclusion, we have shown that the increased production of TGF-β by ATL cells and some HTLV-I-infected cell lines may be related to the transactivation of the TGF-β promoter by the HTLV-I-derived protein, Tax. Using deletion mutants of the TGF-β promoter, we have mapped the Tax-responsive element to the AP-1-binding sites in the TGF-β promoter. The excessive production of TGF-β1 may be important in the pathogenesis of the immunosuppression and hypercalcemia associated with ATL.

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