Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 5389–5393, August 1987
Immunology

Activation of interleukin 2 and interleukin 2 receptor (Tac) promoter expression by the trans-activator (*tat*) gene product of human T-cell leukemia virus, type I

(adult T-cell leukemia/trans-activation/T-cell growth/cyclosporin A)

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Communicated by J. Edward Rall, April 6, 1987 (received for review February 3, 1987)

ABSTRACT **Cotransfection of cDNA encoding the trans-activator gene product of human T-cell leukemia virus, type I (HTLV-I) (*tat*-I), which acts in trans to augment viral gene expression, has revealed strong regulatory effects of this viral protein on the inducible cellular promoters governing human interleukin 2 (IL-2) and IL-2 receptor (Tac) gene expression. The *tat*-I protein stimulates a 3- to 6-fold increase in IL-2 receptor (Tac) promoter activity in transfected Jurkat T cells, but not in the natural killer-like YT cell line, as measured by changes in the expression of the chloramphenicol acetyltransferase (CAT; EC 2.3.1.28) reporter gene linked to this promoter. In contrast, *tat*-I alone has little or no effect on IL-2 promoter activity in Jurkat T cells but markedly synergizes with other mitogenic stimuli (phytohemagglutinin, phorbol 12-myristate 13-acetate, or the OKT3 monoclonal antibody), which alone are ineffective. The *tat*-I protein also partially circumvents the pronounced inhibitory effects of cyclosporin A on the IL-2 promoter. Other cellular and viral promoters are unaffected by the *tat*-I gene product, either alone or in combination with other mitogens. The specific effects of the *tat*-I gene product on the IL-2 and IL-2 receptor (Tac) promoters suggest the possibility of an autocrine or paracrine mechanism of T-cell growth as an early event in HTLV-I-mediated leukemogenesis.**

The human T-cell leukemia virus, type I (HTLV-I), has been implicated as the cause of an aggressive and often fatal neoplasm of $T4^+$ lymphocytes termed adult T-cell leukemia (ATL) (1–3). However, unlike many other acutely transforming animal retroviruses, HTLV-I does not appear to contain a viral oncogene related to a cellular gene (4, 5). Furthermore, insertional mutagenesis by the virus appears unlikely since the site of proviral integration varies markedly in different tumors (6). A striking feature of HTLV-I-infected leukemia T-cell lines is the constitutive high level expression of IL-2 receptors (IL-2R) (7–9) and, rarely, the simultaneous production of IL-2 (10, 11). Speculation has focused on the possibility that the inappropriate expression of these cellular genes, which play an important role in regulating normal T-cell growth, may be involved in the development of ATL (11). However, the mechanism by which HTLV-I alters IL-2R, and occasionally IL-2, gene expression is unresolved. Like some DNA-transforming viruses (12, 13), HTLV-I contains a transactivator (*tat*-I, x-lor, $p40^x$) gene (14) whose 40,000 M_r protein product is able to stimulate transcription of other viral genes by direct or indirect interaction with a trans-activation responsive region located within the viral long terminal repeat (LTR) (15–17). These trans-acting transcriptional properties of *tat*-I raise the possibility that this protein might also be able to alter the expression of certain cellular genes including IL-2R and IL-2. In this regard, Inoue *et al.* (18) have reported that the $p40^x$ (*tat*-I) protein of HTLV-I induces the transient expression of the endogenous IL-2R and IL-2 genes in Jurkat and HSB-2 T cells. We have studied effects of *tat*-I by constructing expression plasmids encoding functional *tat*-I protein or, alternatively, a nontrans-activating protein (Δ*tat*-I) also derived from the pX region of HTLV-I. Effects of these viral gene products on the promoter segments of the IL-2R and IL-2 genes linked to the chloramphenicol acetyltransferase (CAT; EC 2.3.1.28) reporter gene were evaluated in transient cotransfection assays.

MATERIALS AND METHODS

Cells and Reagents. Jurkat, MT-1 (a gift from I. Miyoshi, Kochi Medical School) (19), YT cells (a gift from J. Yodoi, Kyoto University, Kyoto, Japan) (20), and MLA-144 T cells (21) were cultured in RPMI-1640 medium (GIBCO) supplemented with 10% fetal calf serum, 2.0 mM L-glutamine, and antibiotics. In some experiments, phytohemagglutinin (PHA, 1 μg/ml, Burroughs Wellcome, Research Triangle Park, NC), OKT3 monoclonal antibody (100 ng/ml, Ortho Diagnostics), phorbol 12-myristate 13-acetate (PMA, 50 ng/ml, Sigma), and/or cyclosporin A (CSA, 1 μg/ml, Sandoz Pharmaceutical) were added.

Construction of Expression Vectors and Promoter-CAT Plasmids. The plasmids used in these experiments are schematically depicted in Fig. 1. All constructions were made following general procedures (22). The *tat*-I (spFMTLTR/82-2C) and Δ*tat*-I (spFMTLTR/62-6) plasmids were prepared with cDNAs isolated from a λgt10 cDNA library prepared by using poly(A)-enriched mRNA from the HTLV-I-infected C91/PL T-cell line. The *tat*-I cDNA encodes the 40,000 M_r *tat*-I protein as well as a 27,000 M_r protein derived from the X-III reading frame of HTLV-I (data not shown). The Δ*tat*-I cDNA is derived from a mRNA utilizing an alternative splicing pattern within the pX region and does not encode the 40,000 M_r *tat*-I protein but does produce a 21,000 M_r protein derived from the X-III reading frame. Only the *tat*-I cDNA trans-activates the HTLV-I LTR (data not shown). These cDNAs were excised with *Eco*RI and inserted into the unique

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Abbreviations: IL-2, interleukin 2; IL-2R, IL-2 receptor(s) (Tac antigen); HTLV-I, human T-cell leukemia virus, type I; ATL, adult T-cell leukemia; *tat*-I, trans-activator protein of HTLV-I; LTR, long terminal repeat; CAT, chloramphenicol acetyltransferase; PHA, phytohemagglutinin; PMA, phorbol 12-myristate 13-acetate; CSA, cyclosporin A; SV40, simian virus 40; RSV, Rous sarcoma virus; GALV, gibbon ape leukemia virus.

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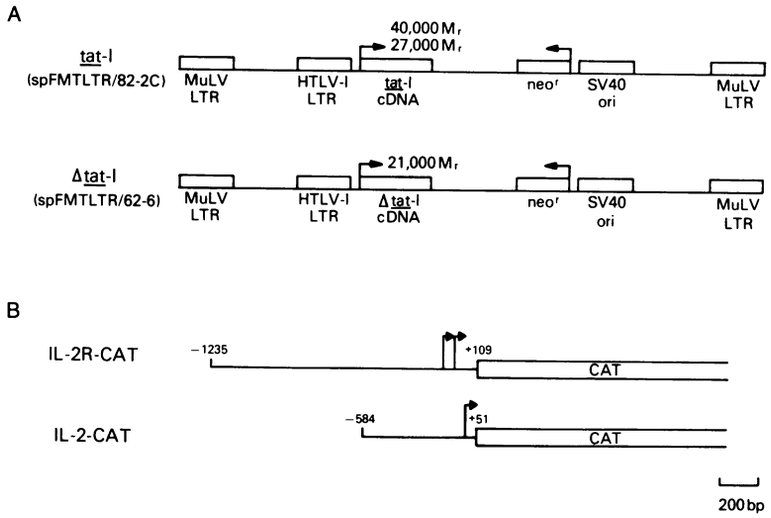

FIG. 1. (*A*) *tat*-I and Δ*tat*-I cDNA expression plasmids. (*B*) IL-2R-CAT and IL-2-CAT reporter plasmids. SV40, simian virus 40; bp, base pairs; MuLV, murine leukemia virus; ori, origin; neo^r, neomycin resistance.

*Eco*RI site of the spFHXneo retroviral expression vector that is derived from a murine Moloney leukemia virus that encodes resistance to the antibiotic G418 under the control of the SV40 early region promoter. In the *tat*-I vector, the *tat*-I protein augments its own synthesis by activating the HTLV-I LTR.

IL-2-CAT (23) contains the 635-bp *Rsa* I fragment from the 5′ region of the human IL-2 gene (−584 to +51 relative to the cap site) (24, 25) inserted into the *Hin*dIII site of JymCAT0. IL-2R-CAT contains a 1.4-kilobase (kb) *Pst* I fragment from the 5′ region of DNA flanking the human IL-2R gene (−1235 to +109 relative to the most 3′ major cap site) (26, 27) inserted into the *Hin*dIII site of JymCAT0 after the formation of blunt ends and the attachment of synthetic *Hin*dIII linkers. *myc*-CAT (a gift of U. Siebenlist, National Institutes of Health) contains the *Hin*dIII–*Pvu* II promoter fragment of the human *myc* gene inserted into the pSV0CAT plasmid. Actin-CAT (pAZ1037, a gift of B. Paterson, National Institutes of Health) contains the chicken β-actin promoter regulating the CAT gene as well as the SV40 enhancer region positioned downstream of the CAT gene (28). RSV-CAT (29), GALV-CAT (30), and visna-CAT (a gift of C. Rosen, Dana–Farber Cancer Institute) contain, respectively, the LTRs of Rous sarcoma virus (RSV), gibbon ape leukemia virus (GALV), and visna virus promoting expression of the CAT gene.

Cotransfection Analyses of Promoter Activity. DNA was transfected into the cell lines by the DEAE-dextran method (30). Five micrograms of the *tat*-expressing or control plasmid and 5 μg of the appropriate promoter-CAT construct were cotransfected into 10^7 cells. PHA, OKT3, and/or PMA were added after 24 hr of culture. Cells were collected for assay after an additional 20 hr of incubation. CAT assays and thin-layer chromatography were performed as described (31).

RESULTS

Comparison of Activation Requirements for Endogenous and Transfected IL-2R and IL-2 Genes in Jurkat T Cells. The Jurkat acute T4$^+$ lymphocytic leukemia T-cell line was used in many of the studies as these leukemia cells share with normal T cells a similar pattern of gene activation following mitogenic stimulation. In the clone of Jurkat T cells that we have used, a single mitogenic stimulus (PHA, PMA, or OKT3) is sufficient to activate IL-2R gene expression. In contrast, these single signals fail to stimulate IL-2 gene expression. Rather, a combination of stimuli (e.g., PHA with PMA or OKT3 with PMA) is required for the activation of the IL-2 gene (refs. 32–36 and data not shown).

The stimulatory effects of these mitogenic signals on the transfected IL-2R-CAT and IL-2-CAT plasmids paralleled their effects on the respective endogenous genes. For example, either PHA or PHA alone produced a 2- to 3-fold increase in expression of IL-2R-CAT in Jurkat T cells (Table 1, line 1) though it produced little or no effect on IL-2-CAT expression (Table 1, line 3). However, a combination of PHA and PMA produced nearly a 60-fold increase in IL-2-CAT expression (Table 1, line 3). In addition, OKT3 synergized with PMA to produce a modest increase in IL-2-CAT expression.

Effect of *tat*-I on pIL-2R-CAT Expression. Potential stimulatory effects of the *tat*-I gene product on the IL-2R promoter were next analyzed. Cotransfection of the *tat*-I expression plasmid produced an ≈6-fold increase in IL-2R-CAT activity compared with the Δ*tat*-I control plasmid (Fig. 2; Table 1, lines 1 and 2). The *tat*-I gene product was also able to activate a 0.6-kb IL-2R promoter fragment (−471 to +109) almost as efficiently as the larger 1.4-kb promoter fragment (data not shown).

Table 1. Effects of *tat*-I and mitogenic stimuli on IL-2R and IL-2 promoter expression in transfected Jurkat T cells

DNA transfected	Inducer					
	Medium	PHA	PMA	PHA + PMA	OKT3	OKT3 + PMA
IL-2R-CAT + Δ*tat*-I	1.0*	2.6 ± 0.03	2.2 ± 0.07	2.9 ± 0.06		
IL-2R-CAT + *tat*-I	6.4 ± 0.09	6.6 ± 0.05	6.6 ± 0.10	6.8 ± 0.13		
IL-2-CAT + Δ*tat*-I	1.0*	2.4 ± 0.48	1.0 ± 0.04	59.3 ± 9.7	1.0 ± 0.05	3.0 ± 0.23
IL-2-CAT + *tat*-I	1.3 ± 0.14	57.9 ± 10.7	20.3 ± 3.2	104.3 ± 17.3	3.3 ± 0.26	11.9 ± 1.2

CAT assays utilized extracts from 2 × 10^6 Jurkat cells that were incubated with [^{14}C]chloramphenicol substrate for 10–12 hr. Results shown represent mean values ± SEM compiled from three to six different experiments.

*Relative CAT activity corresponds to the ratio of % transacetylation obtained in the experimental samples compared with % transacetylation in cells transfected with IL-2R-CAT or IL-2-CAT and the Δ*tat*-I control plasmids in the absence of mitogenic stimuli. The absolute value for % transacetylation for these control samples was as follows: line 1, 1.1%; line 3, 0.9%.

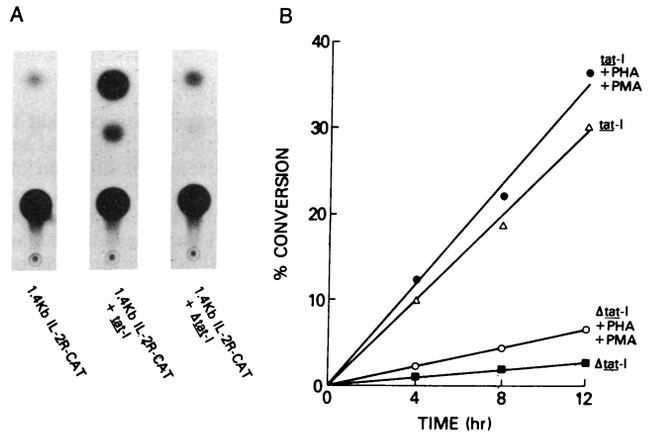

FIG. 2. (*A*) Effects of cotransfection of *tat*-I and Δ*tat*-I plasmid with IL-2R-CAT in Jurkat T cells. (*B*) Kinetic analysis of CAT enzyme activity. Extracts from Jurkat T cells cotransfected and/or induced as indicated were incubated for 4, 8, or 12 hr and % transacetylation of [^{14}C]chloramphenicol was measured.

Cellular Specificity of *tat*-I Induction of pIL-2R-CAT. To explore whether *tat*-I and mitogen-induced responsiveness of IL-2R gene expression were always coordinately linked, other lymphoid cell lines were surveyed in the cotransfection assays (Table 2). As in Jurkat T cells, the IL-2R-CAT plasmid was activated in GALV-transformed MLA-144 T cells by the *tat*-I protein and mitogenic stimuli (PHA and PMA). In contrast, although *tat*-I augmented IL-2R-CAT expression in HTLV-I-infected MT-1 cells (which do not produce detectable levels of *tat*-I protein) (19), mitogenic stimulation of these cells was largely without effect. A different pattern of IL-2R-CAT stimulation was found in the "natural killer-like" YT cell line. Although the addition of PHA and PMA produced marked stimulation of IL-2R-CAT activity in these cells, *tat*-I was completely ineffective.

Effect of *tat*-I on pIL-2-CAT Expression. In contrast to the stimulatory effects on IL-2R-CAT expression in Jurkat T cells, the *tat*-I protein alone was found to have little or no stimulatory effect on IL-2-CAT activity in the absence of other stimuli (Fig. 3; Table 1, line 4). However, when one of a number of different activation signals (PHA, PMA, or OKT3, each of which alone was ineffective) was added in concert with *tat*-I, marked activation of IL-2-CAT expression was produced (Fig. 3). Specifically, the *tat*-I protein, but not the Δ*tat*-I protein, synergized with PHA, PMA, and OKT3 to produce, respectively, a 58-fold, 20-fold, and 3-fold increase in IL-2-CAT activity (Table 1, lines 3 and 4). Although the stimulation with *tat*-I and OKT3 is modest, it is similar to that observed with the combination of OKT3 and PMA.

The *tat*-I Protein Partially Circumvents CSA Inhibition of IL-2-CAT Expression. CSA is a potent immunosuppressive agent that regulates IL-2 activity in Jurkat cells by blocking mitogen-activated transcription of the IL-2 gene (37, 38). Similarly, CSA completely blocked the activation of IL-2-CAT expression induced by PHA and PMA in the cotransfection assays (Fig. 4). In contrast, the inhibitory effects of CSA were partially abrogated by the presence of the *tat*-I protein (Fig. 4). For example, in cells stimulated with *tat*-I in the presence of CSA, 27% of the CAT activity remained after PHA induction, 79% remained after PMA induction, and 47% remained after addition of both PHA and PMA (Fig. 4 and data not shown).

Effects of the *tat*-I Protein on Other Cellular and Viral Promoters. In contrast to the IL-2-CAT and IL-2R-CAT plasmids, expression of other transfected cellular and viral promoters was unaffected by the *tat*-I protein, either when added alone or in the presence of PHA and/or PMA. As shown in Table 3, expression of the *myc* promoter, the RSV LTR, the β-actin promoter linked to the SV40 enhancer, the GALV LTR, or the visna virus LTR was not significantly altered by the *tat*-I protein alone in Jurkat T cells (Table 3). The *myc*, RSV, and actin promoters also were not markedly activated in these cells by PHA and PMA, either in the presence or absence of the *tat*-I protein. In contrast, both the GALV and visna virus LTRs were activated by the mitogenic stimuli. However, the *tat*-I protein did not produce a further increase in their expression.

DISCUSSION

In these studies, we have examined the potential stimulatory effects of the trans-activator gene product (*tat*-I) of HTLV-I on the cellular regulatory sequences governing IL-2 and IL-2R gene expression. We have demonstrated that the *tat*-I protein in Jurkat T cells is able to activate the IL-2R promoter in the absence of any other mitogenic signals. These effects do not appear to simply reflect generalized nonspecific activation, as the *tat*-I protein fails to alter the expression of other cellular and viral promoters (Table 3). In addition, analyses of various lymphoid cell lines revealed that mitogen and *tat*-I induction of the IL-2R promoter were not always correlated (Table 2).

In contrast to its capacity to activate the IL-2R promoter, the *tat*-I protein alone produces little or no stimulation of the IL-2 promoter in Jurkat T cells. The *tat*-I protein, however, is able to synergize with other mitogenic stimuli (PHA, PMA, OKT3), which alone are ineffective, to markedly activate the IL-2 promoter. These costimulatory effects of *tat*-I and mitogen on IL-2-CAT expression also appear to be specific since combinations of these agents do not alter expression of several other cellular or viral promoters. Thus, the *tat*-I protein is able to provide one of the two signals required for IL-2 gene expression and, interestingly, can substitute in this response either for PHA or PMA. The similarity of the ultimate site of action of PHA and PMA predicted by this result is supported by the studies of Fujita *et al.* (39), who have concluded that the same region of the IL-2 promoter is involved in both PMA and concanavalin A induction.

We have also observed that *tat*-I-mediated costimulation of the IL-2 promoter partially circumvents the inhibitory effects of CSA. Though the mechanism of CSA inhibition of IL-2

Table 2. Effects of *tat*-I and mitogenic stimuli on IL-2R-CAT activity in different lymphoid cell lines

DNA transfected	Inducer	Cell line			
		Jurkat	MLA-144	MT-1	YT
IL-2R-CAT + Δ*tat*-I	Medium	1.0*	1.0	1.0	1.0
	PHA + PMA	2.9 ± 0.06	2.5 ± 0.8	1.3 ± 0.07	6.1 ± 1.2
IL-2R-CAT + *tat*-I	Medium	6.4 ± 0.09	8.2 ± 1.7	3.5 ± 0.50	0.7 ± 0.07
	PHA + PMA	6.8 ± 0.13	16.1 ± 7.1	2.8 ± 0.15	6.5 ± 1.7

See legend to Table 1.

*Relative CAT activities: the absolute % transacetylation in the presence of the Δ*tat*-I plasmid and in the absence of mitogenic inducers was as follows: Jurkat, 1.1%; MLA-144, 0.48%; MT-1, 2.7%; and YT, 2.4%.

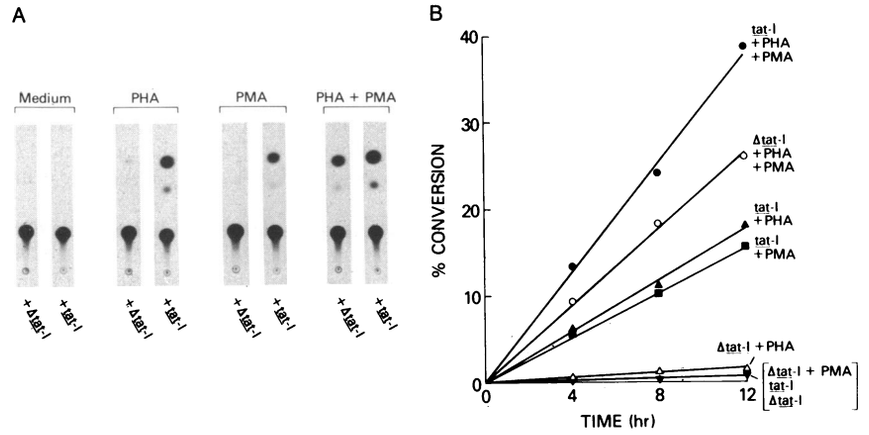

FIG. 3. (*A*) IL-2-CAT expression in Jurkat T cells following cotransfection with the *tat*-I or Δ*tat*-I plasmid in the presence or absence of PHA (1 μg/ml), PMA (50 ng/ml), or PHA + PMA. Mitogenic stimulants were added 24 hr after transfection and CAT enzyme activity was measured 20 hr later. (*B*) Kinetic analysis of CAT enzyme activity in Jurkat T cells transfected with IL-2-CAT in the presence or absence of the *tat*-I or Δ*tat*-I plasmid and mitogenic stimuli (PHA, 1 μg/ml; PMA, 50 ng/ml; or PHA + PMA). The % transacetylation in each of the indicated extracts was determined after 4, 8, and 12 hr of incubation.

gene expression remains unknown, this finding suggests that the *tat*-I protein exerts its stimulatory effects at a point in the activation pathway distal to the site of action of this immunosuppressive drug.

We have also found that the *tat*-II gene product exerts identical though less marked effects on IL-2 and IL-2R promoter-CAT activity (data not shown). Earlier studies with stable cell lines containing the *tat*-II gene had revealed activation of endogenous IL-2 and IL-2R gene expression (40). However, subsequent investigations indicated that the experimental and control cell lines used in these experiments were of different genotype, making firm interpretation of the data impossible (41).

The mechanism by which the *tat* proteins stimulate expression of the transfected IL-2R and IL-2 promoters, as well as the endogenous cellular genes (18), is unclear. Although the *tat*-I protein has been localized to the nucleus (42, 43), it remains unresolved whether this protein is able to bind to DNA. The IL-2 and IL-2R promoters do not share strong sequence homology with the 21-bp enhancer-like region of the HTLV-I LTR shown to be involved in *tat*-I-induced trans-activation (44, 45). It is possible that the *tat*-I protein alters gene expression by interacting with another protein or family of proteins, which, in turn, bind DNA. In this regard, our transfection studies in YT cells strongly implicate an additional cellular factor(s) in the stimulation of IL-2R gene expression by *tat*-I. Although the IL-2R promoter is readily activated in these cells by addition of PMA or PHA with PMA (ref. 20; Table 2), it is completely unresponsive to activation by *tat*-I. This failure is not due to the absence of the *tat*-I protein in the transfected YT cells since the LTR of HTLV-I is markedly trans-activated in these cells. It is possible that the apparent restriction of HTLV-I-induced leukemia transformation to the $T4^+$ subset of T cells reflects the cellular distribution of a putative cofactor required for *tat*-I-induced changes in cellular gene expression.

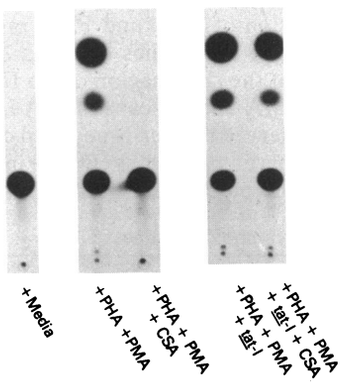

FIG. 4. Effects of CSA on mitogen- and *tat*-I-induced activation of the IL-2 promoter. Jurkat T cells were cotransfected with the IL-2-CAT and Δ*tat*-I plasmids or the IL-2-CAT and *tat*-I plasmids and stimulated with PHA (1 μg/ml) and PMA (50 ng/ml) in the presence or absence of CSA (1 μg/ml).

The finding that the *tat*-I protein is able to activate the IL-2R promoter provides a plausible explanation for the constitutive expression of large numbers of IL-2R on HTLV-I-infected T-cell lines. Furthermore, the ability of the *tat*-I protein to provide one of the two cellular signals required for IL-2 gene expression may also predispose these virally infected T cells to produce IL-2 following antigenic stimulation. Ordinarily, this single signal would be ineffective. The *tat*-I-induced expression of IL-2R and the production of IL-2 could lead to an early period of uncontrolled autocrine polyclonal T-cell growth. The *in vitro* transformation of T cells by HTLV-I is, in fact, characterized by an early stage of vigorous polyclonal T-cell proliferation (46). Such *tat*-I-induced autocrine proliferation might facilitate the occurrence of the second-stage events required for the completion of the T-cell transformation process and lead to the later outgrowth of a clonal population of growth factor-independent leukemia T cells. As *tat*-I activity is often low or undetectable in these IL-2-independent cells, its presence may not be required for maintenance of the transformed state. In support of an autocrine stage in leukemogenesis, Arima *et al.* (47) have identified primary ATL tumor cells whose proliferation, at least in part, involves the simultaneous display of IL-2R and the production of IL-2. It seems likely that the *tat*-I protein plays an important role in the induction of HTLV-I-associated malignancy not only by amplifying viral replication but also by deregulating the expression of the cellular genes that normally control T-cell growth, including IL-2 and IL-2R.

Note. Since submission of this paper, Maruyama *et al.* (48) have reported that the $p40^x$ (*tat*-I) gene product activates expression of CAT plasmids containing the 5′ flanking regions of either the IL-2 or IL-2R genes, even in the absence of mitogen. Unlike the Jurkat T cells we used, the Jurkat T cells employed in those studies appear to produce IL-2 following stimulation with a single signal (concanavalin A).

Note Added in Proof. Cross *et al.* (49) have reported results similar to ours regarding *tat*-I and mitogen activation of the IL-2R and IL-2 promoters.

We thank Drs. C. Rosen and W. A. Haseltine for the gift of the visna-CAT plasmid, Dr. B. Paterson for the gift of the actin-CAT plasmid, and Dr. U. Siebenlist for the gift of the *myc*-CAT plasmid.

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Table 3. Effects of *tat*-I and mitogenic stimuli on other cellular and viral promoters

DNA transfected	Inducer			
	Medium	PHA	PMA	PHA + PMA
myc-CAT + Δ*tat*-I	1.0*	1.0 ± 0.16	0.70 ± 0.11	0.70 ± 0.13
myc-CAT + *tat*-I	1.2 ± 0.31	1.3 ± 0.47	0.95 ± 0.06	0.79 ± 0.01
RSV-CAT + Δ*tat*-I	1.0*	1.2 ± 0.23	1.4 ± 0.31	1.1 ± 0.30
RSV-CAT + *tat*-I	1.0 ± 0.09	1.0 ± 0.26	1.5 ± 0.27	1.1 ± 0.23
Actin-CAT + Δ*tat*-I	1.0*	1.5 ± 0.13	1.4 ± 0.20	1.1 ± 0.15
Actin-CAT + *tat*-I	1.4 ± 0.15	1.5 ± 0.30	1.5 ± 0.16	1.1 ± 0.05
GALV-CAT + Δ*tat*-I	1.0*	4.2 ± 0.75	3.5 ± 0.36	9.0 ± 1.2
GALV-CAT + *tat*-I	0.60 ± 0.01	4.1 ± 0.54	4.7 ± 1.0	9.0 ± 0.80
Visha-CAT + Δ*tat*-I	1.0*	1.0 ± 0.01	2.7 ± 0.93	3.5 ± 2.0
Visna-CAT + *tat*-I	0.92 ± 0.11	1.1 ± 0.05	2.2 ± 0.59	3.3 ± 0.51

See legend to Table 1. Incubations were for 3 hr.

*Relative CAT activities: the absolute % transacetylation in the presence of the Δ*tat*-I plasmid and in the absence of inducer was as follows: *myc*-CAT, 1.0%; RSV-CAT, 8.5%; actin-CAT, 15%; GALV-CAT, 3.3%; and visna-CAT, 1.3%.

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