trans Activation of the Tumor Necrosis Factor Alpha Promoter by the Human T-Cell Leukemia Virus Type I Tax₁ Protein

HUGO ALBRECHT,^{1,2} ALEXANDER N. SHAKHOV,¹ AND C. VICTOR JONGENEEL^{1*}

Ludwig Institute for Cancer Research, Lausanne Branch,¹ and Swiss Institute for Experimental Cancer Research,² CH-1066 Epalinges, Switzerland

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In a cotransfection assay, the human T-cell leukemia virus type I Tax₁ gene product specifically activated transcription from the mouse tumor necrosis factor α promoter. The activation patterns of 5' deletion mutants, artificial enhancer constructs, and point mutations in the promoter indicate that the major Tax₁-responsive element is a site at position –655 which binds the NF- κ B/rel and NF-GMa transcription factors.

Human T-cell leukemia virus type I (HTLV-I) is thought to be the etiological agent of at least two surprisingly different pathologies: adult T-cell leukemia, an aggressive T-lymphocyte malignancy, and tropical spastic paraparesis, a neurodegenerative disease accompanied by severe myelopathy (8). Although the precise mechanisms by which HTLV-I can cause these pleiotropic effects remain unknown, much attention has focused on one viral gene product, Tax₁, a transcriptional modulator which up-regulates the expression of viral genes and also affects a large number of host genes (23). In particular, the Tax₁-induced constitutive expression of the interleukin 2 receptor α chain in HTLV-I-infected T lymphocytes is thought to play a central role in their uncontrolled proliferation (5, 7, 10). Tax₁ has also been shown to induce the synthesis of many proteins that may contribute to the pathology of HTLV-I infections (9, 13, 14, 16, 22, 25). HTLV-I-infected cell lines have been reported to constitutively secrete tumor necrosis factors alpha and beta (TNF- α and TNF- β) (24). Therefore, it was of interest to determine whether Tax₁ could activate the TNF- α promoter in macrophages and, if so, through which *cis*-acting elements it may act.

A construct containing 1,059-bp of TNF- α upstream sequence coupled to the chloramphenicol acetyltransferase (CAT) reporter gene [TNF(–1059)CAT] is silent when transfected into bone marrow-derived macrophages but can be strongly activated upon exposure of the cells to bacterial lipopolysaccharide (LPS) (18). When we cotransfected 4 μ g of the pcTAX plasmid (15) (Fig. 1) with TNF(–1059)CAT, we observed an activation approximately equal in magnitude to that caused by 100 ng of LPS per ml (Fig. 2). The addition of LPS failed to increase the magnitude of the signal triggered by pcTAX (data not shown). To make sure that the activation which we observed was indeed induced by the Tax₁ protein, we cotransfected the TNF(–1059)CAT plasmid with two derivatives of pcTAX, pcTAX Δ NcoI and pcTAX Δ Sma/Bam. In the first construct, the *tax*₁ initiation codon was selectively mutated, while in the second the *tax*₁ coding sequence had been removed entirely (Fig. 1). Cotransfection with either pcTAX Δ NcoI (Fig. 2) or pcTAX Δ Sma/Bam (data not shown) failed to activate either

the TNF(–1059)CAT plasmid or its 5' truncated derivatives. Therefore, it is likely that the Tax₁ protein is directly responsible for the transcriptional activation of the TNF- α promoter.

To obtain a rough localization of the *cis*-acting elements in the TNF- α promoter that may be responsible for Tax₁-mediated activation, we cotransfected a series of 5' deletion mutants of the TNF- α promoter with the pcTAX plasmid. The largest drop in Tax₁-mediated activation occurred between positions –695 and –655 (Fig. 2), the mean difference between the activities of the two constructs being about 10-fold (data not shown). Since the TNF(–655)CAT construct had lost part of one of the NF- κ B-binding sites found in the mouse TNF- α promoter (κ B2), we suspected that this site might play a role in Tax₁-mediated activation.

We tested, for activation by Tax₁, a number of constructs containing NF- κ B-binding elements of the TNF- α promoter inserted upstream of an enhancerless herpes simplex virus thymidine kinase promoter. Constructs containing multiple copies of the κ B3 element, which had been previously identified as a strong LPS-responsive element (4, 18), were also activated by Tax₁ (Table 1). In contrast to the results obtained with LPS, a construct containing only one copy of the κ B2 element was also strongly activated by Tax₁ (Table 1). These results establish the κ B2 motif as a strong Tax₁-responsive enhancer. To further assess the seemingly central role of site κ B2 in Tax₁-mediated activation, we tested the effects of point mutations in the TNF- α promoter which selectively destroy individual NF- κ B-binding sites. Mutations of sites κ B1 and κ B3 had only moderate effects on activation by Tax₁, while mutation of site κ B2 had a very profound effect, reducing it approximately 30-fold (Table 2). These results indicate that site κ B2 is the principal Tax₁-responsive element in the mouse TNF- α promoter.

We conclude that Tax₁, the transcriptional activator encoded by HTLV-I, is capable of activating transcription from the TNF- α promoter. This provides a mechanistic explanation for the reported synthesis of TNF- α by HTLV-I-transformed lymphoid cell lines (24). It also points toward TNF- α as another potential agonist in the pathology of HTLV-I infections. TNF- α is known to induce bone resorption and hypercalcemia (2), both of which are prominent features of adult T-cell leukemia (23), and there are reasons to believe that production of TNF- α within the central

* Corresponding author. Electronic mail address: victor.jongeneel@isrec.arcom.ch.

FIG. 1. Structures of the genetic elements used in this study. (A) pX mRNA from HTLV-I (17). The pcTAX plasmid contains the entire region shown as a thick black line downstream of a cytomegalovirus early promoter and has a mutation in the *rex* initiation codon (15). The *Nco*I site used to mutate *tax1* and the *Bam*HI and *Sma*I sites used to excise most of its coding sequence are indicated. (B) Upstream region of the mouse TNF- α promoter. The positions of the four major κ B-like elements of the (CA) $_n$ microsatellite and of the promoter-proximal conserved SP-1 and TATA motifs are indicated.

nervous system could contribute to the demyelination seen in multiple sclerosis and tropical spastic paraparesis (6, 21). There are strong indications that Tax $_1$ can induce the production of TNF- β by lymphoid cells and that this activation is mediated through a κ B-like element located immediately upstream of the TNF- β promoter (12, 14). Since the biological activities of the two forms of TNF are extremely similar, the effects of Tax $_1$ on both promoters could lead to cumulative biological effects.

The Tax $_1$ protein has been shown to act principally on two classes of enhancers, those of the κ B and of the ATF/CREB families (23). Both classes of enhancers can bind multiple factors with related recognition specificities, making it difficult to correlate activation by Tax $_1$ with the activity of a

unique transcription factor. The main mediator of Tax $_1$ activation through κ B-type enhancers has been reported to be NF- κ B itself (a heterodimer of 50- and 65-kDa subunits) or a complex containing the 86-kDa product of the *c-rel* gene (1, 11, 23). Our data indicate that the principal *cis*-acting element involved in Tax $_1$ -mediated activation of the mouse TNF- α promoter is site κ B2. We have shown previously that this site binds members of the κ B/rel family only weakly but has a high affinity for NF-GMa (20), a transcription factor reported to be involved in the regulation of several cytokine genes (3, 4, 19, 20). Whether Tax $_1$ acts preferentially on other promoters through κ B sites that are also binding NF-GMa remains an open question. Sequence conservation considerations provide additional evidence for an important role of the κ B2 site in TNF- α gene regulation: in all five mammalian species for which the TNF- α upstream region has been sequenced so far (human, mouse, rabbit, rat, and pig), the κ B2 motif (GTGAATTCCC) is perfectly conserved, while lying in a region with an otherwise low level of interspecies similarity (18). It is extremely unlikely that such a strong interspecies conservation of a protein-binding site would be fortuitous.

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FIG. 2. Effects of LPS and Tax $_1$ on the expression of constructs containing TNF- α or simian virus 40 promoters. Bone marrow-derived macrophages were transfected with plasmids containing the CAT gene under the control of 5' truncated TNF- α promoters or the simian virus 40 promoter. Activation was induced by overnight exposure to 100 ng of LPS per ml or by cotransfection with wild-type or mutated pcTAX, and CAT activity was measured as described in the text.

TABLE 1. Tax $_1$ -inducible enhancer activities of κ B-like elements of the TNF- α promoter

No. of copies of:		Activity (% wt) ^a
κ B2	κ B3	
1	0	51 $\pm$ 38
3	0	44 $\pm$ 6
1	1	93 $\pm$ 20
0	6	272 $\pm$ 168

^a pBLCAT2-derived plasmids containing the indicated κ B-like elements were transfected into primary macrophages and assayed for inducibility by Tax $_1$. The values shown (means $\pm$ standard deviations) were derived from at least three experiments using at least two different DNA preparations and were adjusted for the activity obtained with a wild-type promoter [TNF(-1059)CAT] in the same experiment.

TABLE 2. Effects of mutations in κ B-like motifs of the TNF- α promoter on inducibility by Tax₁

Site	Mutation	Activity (% wt) ^a
κ B1	GGGGAATCCTT→CTGTAGTCCTT	33 ± 17
κ B2	GTGAATTCCCA→GTGAATTAACA	3 ± 2
κ B3	GGGGGCTTTCC→GGAAGCTTTCC	22 ± 16

^a Derivatives of TNF(-1059)CAT with the indicated mutations were analyzed for inducibility by Tax₁ in the same manner as described in the footnote to Table 1. Boldface letters indicate the mutated positions in the constructs.

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