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Regulation of the GATA3 Promoter by Human T-Cell Lymphotropic Virus Type I Tax Protein

Simone Cristina Olenscki Gilli,[1] Tereza Sueko Ide Salles,[1] and Sara Teresinha Olalla Saad[2]*

[1]Hematology and Hemoterapy Center, State University of Campinas, Department of Clinical Medicine, Campinas, São Paulo, Brazil
[2]Department of Internal Medicine, State University of Campinas, Department of Clinical Medicine, Campinas, São Paulo, Brazil

Abstract The Human T-cell leukemia virus type I (HTLV-I) non-structural protein Tax plays a crucial role in cellular transformation. It activates the transcription factors of various cellular genes and interacts with cellular proteins. There is limited data available on the interaction between specific T-cell transcription factor GATA3 and Tax. Implications for the significance of GATA3 in T-cell development and function, T helper2 (Th2) differentiation, and a role of GATA3 during the immune response have been reported. To determine the effect of the Tax protein on *GATA3* gene expression, we investigated the interaction between this protein and the GATA3 promoter and repressor regions. Results demonstrated an interaction between Tax and the GATA3promoter via the transcription factor Sp1 and a role for Tax in the negative regulation of GATA3 expression, through its interaction with the repressor ZEB. This interaction may be involved in the pathophysiology of adult T-cell leukemia/lymphoma (ATL) and tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM). J. Cell. Biochem. 93: 1178–1187, 2004. © 2004 Wiley-Liss, Inc.

Key words: GATA3 activity; Tax; viral transactivator; Sp1; ZEB

Human T-cell leukemia virus type I (HTLVI) is an oncovirus of the Retroviridae family [Murphy and Kingsbury, 1990] and was the first retrovirus described following its isolation in the early 1980s from patients with adult T-cell leukemia/lymphoma (ATL) [Poiesz et al., 1980]. HTLV I was subsequently shown to be clinically associated with ATL [Yoshida et al., 1984], as well as with tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM) [Gessain et al., 1985], and a number of chronic diseases, including uveitis, arthritis, and infective dermatitis [Khabbaz et al., 1993]. HTLV-I infections are endemic in high-risk groups in Melanesia, Japan, the Caribbean, sub-Saharan Africa, and the United States. It is currently estimated that 15–20 million individuals worldwide are infected with HTLV-I, and up to 10% of these infected individuals will develop ATL or TSP/HAM [Gessain, 1996; Zucker-Franklin and Pancake, 1998]. The observation that the Caribbean strains of HTLV-I are more easily transmitted and cause disease at a much earlier age than the Japanese strains suggests that HTLV-I may be a more dangerous virus, as reported by the Center for Disease Control in the USA [Satcher, 1995; Ewald, 1996].

The viral non-structural protein, Tax, plays a crucial role in cellular transformation and has multiple functions. It activates, for example, the transcription factors of various cellular genes, such as proto-oncogenes (*c-fos*, *c-jub*, *fra-1*, and *c-myc*) and genes encoding growth factors (IL-2, IL-6, transforming growth factor-β, OX-40 ligand, and granulocyte–macrophage colony-stimulating factor) and their receptors (IL-2 receptor α-chain and OX-40) and represses the transcription of several cellular genes, such as those encoding DNA polymerase β and Bax. Tax also stimulates the activity of several kinase

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*Correspondence to: Sara Teresinha Olalla Saad, MD, PhD, Hemocentro, UNICAMP, Cidade Universitária "Zeferino Vaz", C.P. 6198, Campinas, São Paulo, Brazil, CEP. 13083-970. E-mail: sara@unicamp.br

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enzymes such as cyclin-dependent kinase (CDK) and protein kinase C [Iwanaga et al., 1999]. To perform these activities, Tax interacts with various cellular proteins such as the transcription factors, SRF (CarG binding protein), and CREB (cyclic AMP-responsive element CRE-binding protein), IκB (an inhibitor of NF-κB/Rel transcription factors), MEKK1 (kinase of IκB kinase) and INK-4, an inibitor of CDK [Fujii et al., 1992; Zhao and Giam, 1992; Suzuki et al., 1993; Watanabe et al., 1993; Suzuki et al., 1996; Yin et al., 1998]. These interactions mediate the Tax-dependent activation of transcription via CRE (CREB), CarG (SRF), κB elements (IκB), and CDK kinase (INK-4). Tax also associates with other cellular proteins such as mitotic checkpoint protein MAD-1 and cyclin D [Jin et al., 1998; Neuveut et al., 1998] and remains the major focus of study for further understanding of the mechanism by which HTLV-I transforms human T-lymphocytes. There is, however, limited data available regarding the interaction between the specific T-cell transcription factor, GATA3, and Tax. We had previously demonstrated decreased GATA3 mRNA expression in HTLVI infected individuals and in patients with ATLL and HAM/TSP [Gilli et al., 2000], however, the mechanism involved in this phenomenon was not clarified in the study.

GATA3 is the third member of the GATA family of proteins and has an abundant expression in a number of distinct sites during development, including the kidney, central and peripheral nervous systems, and T-cell compartment [George et al., 1994]. Mice deficient in GATA3 exhibit multiple physiological abnormalities during development and die on embryonic day 12 [Pandolfi et al., 1995]. Along with T-cell ontogeny, GATA3 is detected in a thymic rudiment on embryonic day 12.5 and is expressed throughout mature peripheral T-cells [Oosterwegel et al., 1992; Hattori et al., 1996].

The *GATA3* gene expression pattern, regulated during development and cellular differentiation, may be mediated by a complex array of *cis*-acting elements, as shown in the regulation of transcription factor genes in invertebrates [Arnone and Davidson, 1997]. Two transcriptional control elements were found in the *GATA3* gene, one in the first intron and the other at 8.3–5.9 kb 5′ from the GATA3 transcriptional initiation site. The first intron acts as a strong transcriptional activator with no cell-type specificity. The upstream regulatory element could confer T-cell specificity to the GATA3 promoter activity and the analysis of this region revealed a 707-base pair silencer that drastically inhibited GATA3 promoter activity in non-T-cells. Two CAGGTG E-box could bind USF proteins, the ubiquitious repressor ZEB, or the basic helix-loop-helix proteins E2A and HEB [Gregoire and Romeo, 1999]. The transcriptional control that regulates *GATA3* gene expression involves the ZEB repression in the human GATA3 promoter activity in non-T-cells and this effect can be displaced in T-cells.

The significance of GATA3 in T-cell development and function was suggested by the identification of binding sites for GATA3 in the TCR-α, -β, and -δ enhancers [Leiden, 1993], the CD8α promoter/enhancer [Landry et al., 1993], the CD4 enhancer [Wurster et al., 1994], the interferon- δpromoter [Penix et al., 1993], and in the IL-5 promoter [Siegel et al., 1995]. More recently, a pivotal role for GATA3 in CD4+ T helper2 (Th2) differentiation has been demonstrated [Zheng et al., 1995; Zhang et al., 1997; Ouyang et al., 1998]. There is, however, limited data available regarding the role of GATA3 in the immune response in animal models, due in part to the embryonic lethality of GATA3 deficiency in mice, which precluded in vivo studies. To determine the effect of the Tax protein in *GATA3* gene expression, the interaction between this protein and its promoter and repressor regions was studied.

We postulated that the transcription of GATA3 might be deregulated by the viral product, Tax, as previously described for other transcription factors. This study was undertaken to investigate the effect of Tax upon the GATA3 promoter and the GATA3 repressor using DNA–protein interaction and "in vitro" transcription assays.

MATERIALS AND METHODS

EMSA

The human GATA3 promoter contains multiple potential binding sites for the transcription factor Sp1. Two sites were studied, using the oligonucleotides containing the Sp1 binding motifs (underlined sequences) in the region −188 to −174 (5′-CCGGTCAGTGGAGGGGC-GGGAGGAG-3′) and region −161 to −129 (5′-CGGGGGTGCGCGGGCGGGGGGAGAAGTC-

CTG-3′) GenBank Accession Number X73519. A probe containing a mutation of the promoter for each region, 5′-CCGGTCAGTAATGACGTAC-TTGAGGAG-3′ and 5′-CGGGGGTGTAGCTCT-TACAGGGGAGAAGTCCTG-3′, was used as a negative control. For the study of the gene repressor region, the oligonucleotide sequence, containing a ZEB motif (5′-AGCTTTTTACCAG-GTGGTCTCT-3′), region −7121 to −7130, was used (GenBank Accession number AJ131811). For the competition assay, a 100-fold molar excess of cold probe was used as well as a negative control, a probe, 5′-AGCTTTAATTCG-TAACGTCTCT-3′, containing a mutation of the promoter ZEB binding region. A radiolabeled double-strand oligonucleotide was prepared as a probe by annealing and filling the overhang, using the Klenow fragment of DNA polymerase I, in the presence of the γ-^{32}P-deoxyadenosine triphosphate. The labeling probes were purified in a G25 sepharose column, centrifuged for 5 min at 2,500 rpm and diluted to 0.5×10^4 cpm.

The purified Tax protein and Tax antibody were a kind gift from Dr. Susan Marriot (National Cancer Institute, Bethesda, MD). The Tax protein was obtained by the expression of a construct containing the entire HTLV-I inserted into a constitutive bacterial expression vector [Giam et al., 1986] and purified as described by Lindoholm et al. [Marriott et al., 1991]. The ZEB antibody was kindly donated by Dr. Thomaz Brabletz (University of Erlangen-Nürnberg, Erlangen, Germany). Tax protein (0.9 μg) and Sp1 (1 footprinting unit) or ZEB (1 μg) were incubated with the labeled probe in a total volume of 14 μl in binding buffer composed of 40 mM Tris-HCl (pH 7.9), 100 mM NaCl, 0.2 dithiotheitol, and 10% glycerol in the presence of 1.6 μg/μl polydeoxyinosinic-deoxycytidilic acid (Pharmacia, Piscataway, NJ) for 15 min. In competition studies, a 100-fold molar excess of cold oligonucleotides were preincubated with Tax protein and Sp1 or ZEB for 15 min at room temperature, prior to addition of the labeled probe. For supershift experiments, anti-Tax or anti-ZEB antibody was added to the reaction mixture and incubated for 15 min.

Samples were subjected to electrophoresis at 240 V for 1.5 h at room temperature in 4% native polyacrylamide gels (acrylamide/bisacrylamide: 30/1) with 0.5 × TBE (44.6 mmol/l Tris base, 44.4 mmol/l boric acid, and 1.0 mmol/l EDTA).

ZEB Protein Expression

Expression constructs encoding the C-terminal zinc-finger 893–1125 human ZEB were kindly provided by Dr. Thomaz Brabletz (University of Erlangen-Nürnberg, Erlangen, Germany). The GST fusion protein was prepared from IPTG-induced *E.coli* DH5α harboring the expression construct. Cells were lysed by a freeze-thaw procedure. Bacterial lysates were incubated with glutathione-sepharose 4B (Amersham Biosciences, Roosendaal, Netherlands) at 4°C with gentle agitation for 30 min. The protein bound sepharose was washed repeatedly, and GST fusion protein was eluted in reduced glutathione buffer. Protein was analyzed by SDS–PAGE (4% gel) and protein concentration was measured using the Bradford assay (Bio-Rad, Richmond, CA). Fractions were stored at −80°C.

Cell Culture

Human Jurkat T-cells were obtained from the American Type Culture Collection (Rockville, MD). The cell lines were grown in RPMI 1640 medium (Gibco-BRL/Life technologies, Inc.) supplemented with 10% fetal bovine serum, 100 U penicillin per ml, 100 μg streptomycin per ml and 2 mM L-glutamine. All cultures were maintained at 37°C and 5% CO_2 in a humidified incubator.

Plasmids

The construct containing the −96/+598 region of the human GATA3 promoter upstream of the *pBLCAT3* reporter gene was kindly provided by Dr. Paul-Henri Romeo (Hopital Henri Mondor, Creteil, France). The CMV-based expression vector containing the coding sequence for wild-type HTLV-1 Tax and the Tax-derived mutant CMV-V89A were kind gifts from Dr. Chou-Zen Giam (Uniformed Services University of Health Sciences, Bethesda, Maryland) [Harrod et al., 1998]. The empty plasmid pBLCAT3 was purchased from the American Type Culture Collection (Rockville, MD).

pSIL-GATA3, the GATA3 promoter silencer vector, was prepared by subcloning the DNA coding the sequence of the human *GATA3* silencer gene amplified by PCR, using the primers SIL1 (5′-GCCCTGAGCATTGTTTGG-TAG-3′; positions −7928 to −7906) and SIL2 (3′-CTAGGAGCTGGAGTGTGATTG-5′; positions −6942 to −6963). PCR was performed in a thermal cycler (Perkin Elmer Thermo-cycler,

Connecticut) for 35 cycles; denaturation at 94°C for 2 min, annealing at 54°C for 60 s and extension at 68°C for 2 min. PCR product was purified using a QIA quick Gel Extration kit (Qiagen, Tokyo, Japan). The isolated DNA fragment was ligated into the TA vector. After transformation of *E.coli* DH5α, the recombinant clones were randomly chosen and the plasmid DNAs were miniprepared using a QIAprep Spin Miniprep kit (Qiagen, Tokyo, Japan). The PCR product was digested with Xba I and Hind III and finally cloned upstream in the construction containing the −96/+598 region. The identity of the inserts was checked by automated DNA sequencing (ABI 377, Applied Biosystem).

Transfection and Cat Assay

Jurkat cells 1×10^7 were resuspended in 400 μl RMPI without serum and were transiently transfected by electroporation in a Bio-Rads Gene Pulser (975 μF and 0.25 kV) with 20 μg −96/+598pBLCAT3 or pSIL-GATA3, plus 10 μg CMV wild-type Tax, CMV-V89A or pBLCAT3. After electroporation, the cuvette was placed on ice for 5 min. The cells were transferred from the cuvette into a flask and fresh medium was added. After 48 h, cells were harvested and CAT ELISA obtained the CAT activity, according to the manufacturer's instructions (Roche Molecular Biochemicals, Mannheim, Germany). Due to transactivation of commonly used viral promoters (SV40, cytomegalovirus, and Rous sarcoma virus) by HTLV-1 Tax, in Jurkat cells, it was difficult to design an internal control and, accordingly, CAT activities were normalized by protein amount (using a copper-based assay Pierce, Rockford, IL) of each extract and presented as relative values by defining the empty plasmid pBLCAT3 as 1.0. Data are means ± standard deviations (bars) values for minimum three independent experiments performed in triplicate.

RESULTS

Tax Promotes Sp1 Binding on the GATA3 Promoter

A clear involvement of Sp1 in the *GATA3* gene regulation has never been determined and footprinting analysis of the GATA3 region −198 to −56 (+1 as the major transcriptional start site) failed to reveal any sequence specifically protected by nuclear extracts from T-cell lines [Labastie et al., 1994]. However, the human GATA3 promoter contains multiple potential binding sites for the transcription factor Sp1 and it has been shown that certain viral and cellular genes bind to this cellular transcription. In this study, we demonstrated an interaction between Tax protein and Sp1 on the GATA3 minimal promoter regions through EMSA (Fig. 1A). The results showed that Sp1 (Fig. 1B), as well as Tax, are unable to bind alone on the GATA3 promoter. In contrast, when Sp1 is added with Tax to the reaction, one major complex (C1) can be detected. The specificity of this interaction was demonstrated through anti-Tax antibody addition, a negative control assay and by competition studies.

Tax Interacts With ZEB in the GATA3 Promoter Repressor Motif

To test the possibility that the *GATA3* gene expression could be repressed by Tax, we investigated whether the Tax protein could interact with the ubiquitous ZEB repressor on the *GATA3* gene silencer (Fig. 2). ZEB actively represses transcription of the human GATA3 promoter by binding to the silencer in the −7828 and −7746; −7197 and −7121 region [Gregoire and Romeo, 1999].

As shown in Figure 2A, high levels of the C1 complex were detected when ZEB and Tax protein were added to the reaction with the silencer region probe. A 100-fold excess of unlabeled ZEB binding oligonucleotides provided an efficient competition. A negative oligonucleotide probe control confirmed the specificity of this interaction. Supershift assays confirmed that the complex contained ZEB and Tax, since the anti-ZEB and anti-Tax antibodies induced a slow migration complex when added to the reaction. ZEB protein binds to the GATA3 silencer and this interaction was confirmed by addition of anti-ZEB (Fig. 2B).

Tax Induces a Modest Activity of the GATA3 Minimal Promoter

In the next series of experiments, we performed transient transfections of the *GATA3* gene minimal promoter, in the Jurkat cell line, to analyze whether the interaction between Tax protein and Sp1 affects the activity of the GATA3 minimal promoter. For this purpose, we used a CAT reporter plasmid carrying the GATA3 minimal promoter [Gregoire and Romeo, 1999]. The reporter plasmid was introduced into Jurkat cells along with a wild type

Tax expression vector, or CMV-V89A Tax mutant expression vector. This mutant compromised the ability of Tax to bind to the CBP/p300 [Harrod et al., 1998]. The cell lysate was prepared 48 h post-transfection and examined for CAT activity, to monitor the promoter expression. Tax protein induced a twofold increase in GATA3 promoter activity, compared with that of cells without Tax. Tax mutant CMV-V89A did not suppress this effect (Fig. 3). The reproducibility of the experiments was high and the results obtained for the transfected group with the construction only were always extremely different to the transfected group with the construction plus the protein expression vector, suggesting that the Tax wild-type and Tax derived mutant proteins were efficiently expressed during transient assays in Jurkat cells.

Tax Represses the GATA3 Promoter Activity in the Presence of the Silencer Region

After demonstrating the ability of Tax to form a complex with the ZEB repressor on the

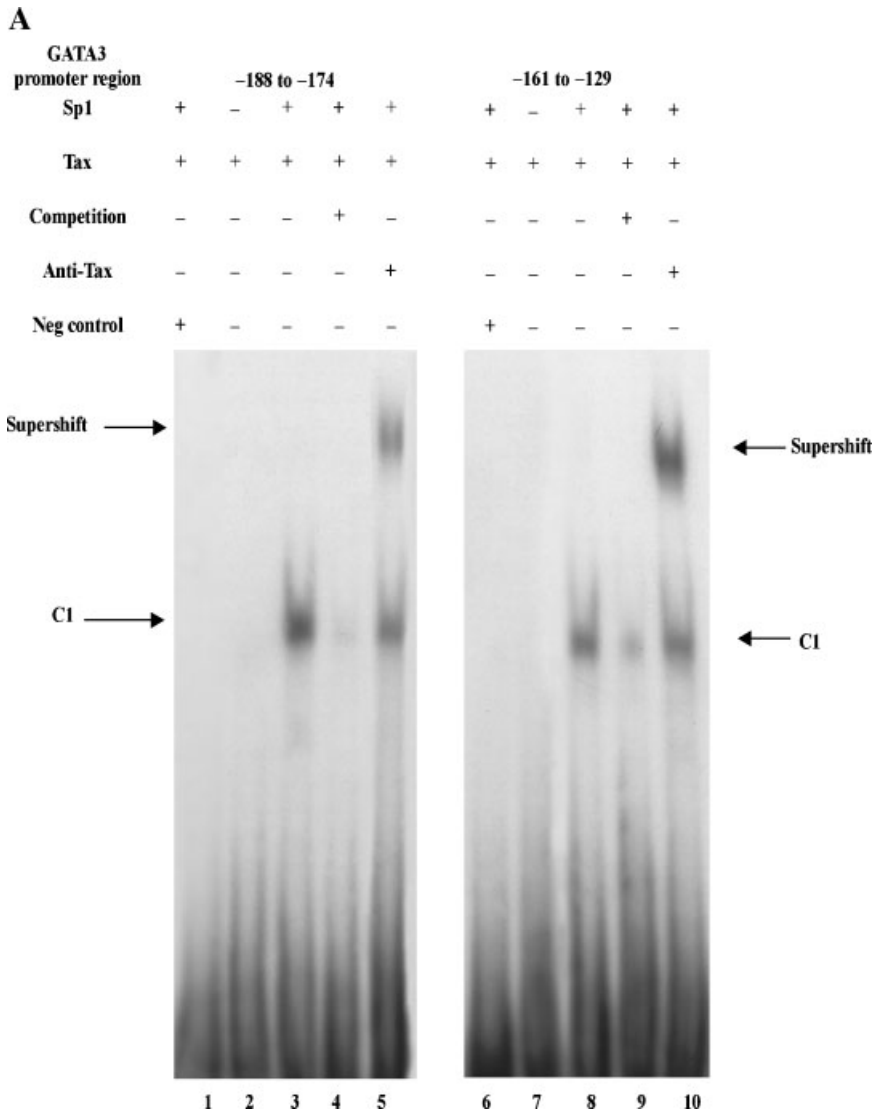

Fig. 1. Tax promotes Sp1 binding on the GATA3 promoter. **A**: The Tax and Sp1 transcription factor was submitted to EMSA using two regions of the GATA3 promoter as a probe. No complex was formed between Tax and the probe (**lanes 2** and **7**). A complex (C1) was observed when Sp1 and Tax protein were added (**lanes 3** and **8**) and a 100-fold molar excess of the cold oligonucleotide inhibited this complex (**lanes 4** and **9**). A negative control inhibits the complex in both promoter regions (**lanes 1** and **6**). Antisera were incubated with Tax protein for 15 min after the beginning of the reaction and a supershift band was observed above the bands (**lanes 5** and **10**). **B**: No complex was formed between Sp1 and the probes (probe 1 correspond to region −188 to −174 and probe 2 correspond to region −161 to −129: **Lanes 1** and **2**).

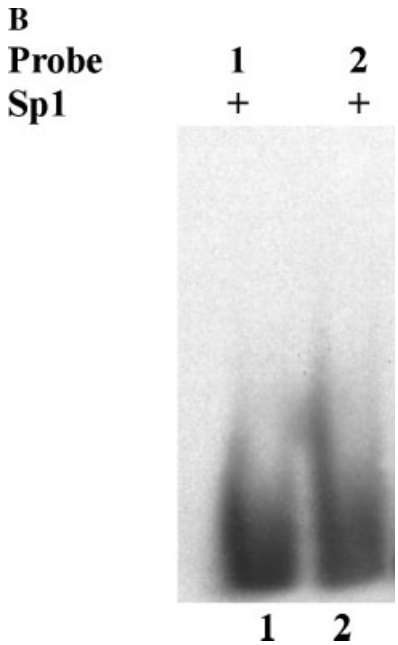

Fig. 1. (*Continued*)

silencer of *GATA3* gene, experiments to elucidate whether this interaction has any repressive action on *GATA3* gene expression were carried out. A segment of the *GATA3* gene, encompassing the repressor region from nt −7828 to −7121, and subcloned upstream contiguous to the sequence −96 to +598, corresponding to the *GATA3* gene minimal promoter (pSIL-GATA3), was co-transfected with a Tax expression vector or its mutants. As expected, an activity of the silencer in T-cells was observed. Whilst this activity was higher than the activity of the construction with the promoter, this difference was relatively small and, therefore, not relevant. Conversely, the pWTax vector reduced CAT expression by 4.9-fold, and this effect was not observed with the V89A mutant (Fig. 4). Again, the reproducibility of the experiments was high and the results obtained for construction only and construction plus the protein expression vector were always extremely different

DISCUSSION

Similar to other viruses, such as simian virus 40 (Tag), adenovirus (E1a), herpes simplex virus (VP16), and human immunodeficiency virus (Tat), HTLV-1 encodes a 40-kDa nuclear protein, Tax, which stimulates transcription from three 21-bp repeat sequences in the viral enhancer. The key element in the 21-bp repeat responsible for Tax activation is the sequence motif, TGACG (T/A) [Jeang et al., 1988], which appears in the enhancer/promoter region of numerous cellular and viral genes. The molecular mechanisms through which Tax affects these transcriptional factors are still under investigation. Tax impinges upon several cellular signal-transduction pathways, including those for CREB/ATF and NF-κB [Jeang, 2001]. In addition to its function as a modulator of cellular transcription, Tax may play a role in the stimulation of host cell proliferation, employing both down- and up-regulation of several proteins. Tax protein affects the expression of several genes relevant to growth, genes encoding proto-oncogenes, the α chain of the IL-2 receptor, cytokines, cyclin D2, and the CDK inhibitor $p21^{CIP}$ and interferes directly with the function of cell cycle regulatory proteins, such as cyclin D1/cyclin D3. In the presence of Tax, the CDKs—CDK4 and CDK6 are activated, suggesting that this viral protein is involving I CDK4/CDK6 stimulation. The CDK4 stimulation may essentially contribute to mitogenic and immortalizing Tax effects [Haller et al., 2002]. Tax has also been shown to be capable of activating promoters when fused to a DNA-binding domain and to bind to the basal transcription factors, TFIIA and TATA-binding protein.

We have previously demonstrated, by semi-quantitative RT-PCR, decreased expression of GATA3 mRNA in HTLV-I carriers and in patients with HTLV-I related diseases [Gilli et al., 2000]. Based on the knowledge of the effects of Tax, we investigated the role played by Tax in GATA3 transcription. Tax induced a modest activity of the GATA3 minimal promoter, however, a great effect was observed in the repressor region. The repressor region of the *GATA3* gene has been described as presenting binding sites for the ZEB protein [Gregoire and Romeo, 1999]. ZEB and E2A/HEB have been shown to regulate genes during hematopoesis. In humans, ZEB represses GATA3 promoter activity, and the E2A/HEB heterodimer can displace ZEB in T-cells. We postulate that the down-regulation of the *GATA3* gene by Tax occurs through ZEB and that this down-regulation might impair or antagonize the E2A/HEB effects in T-cells.

It is possible that the negative interference of Tax in GATA3 expression, described herein, may be a viral tool against the cellular immune

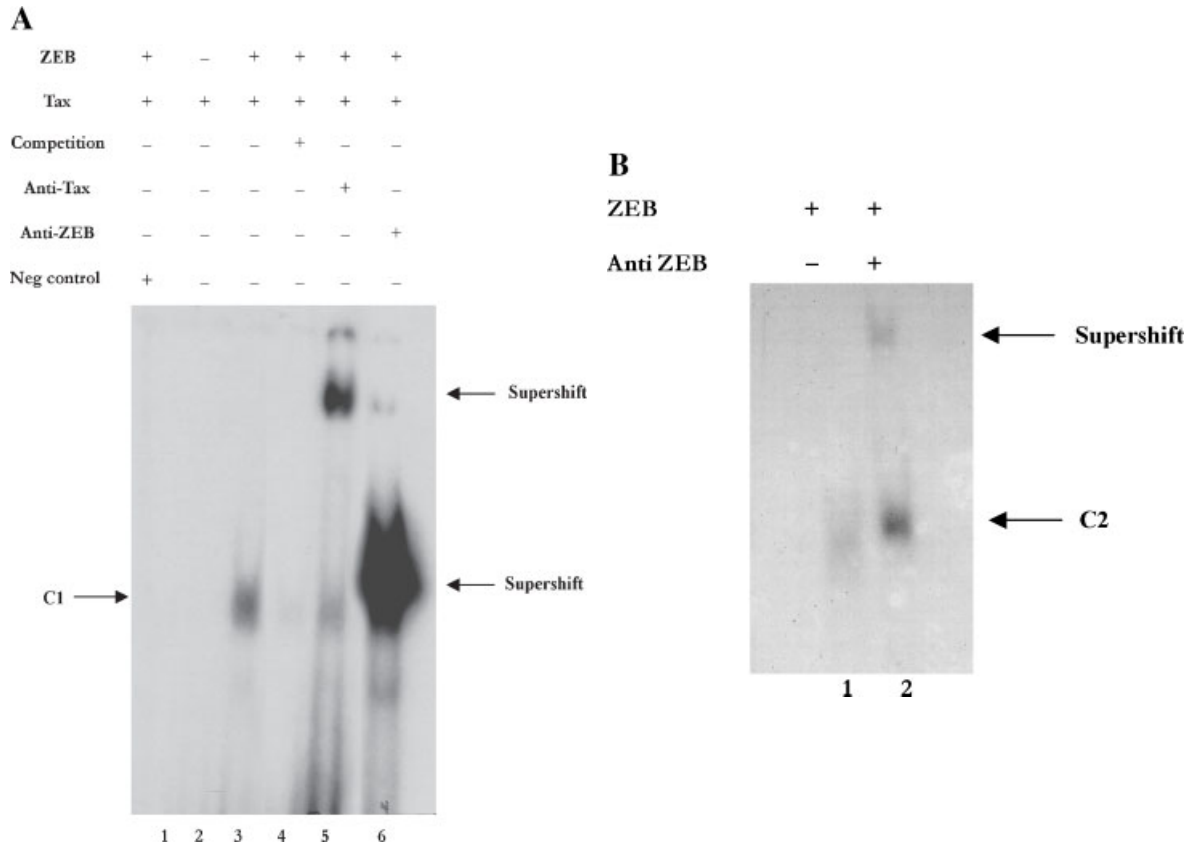

Fig. 2. Tax interacts with ZEB on the GATA3 promoter repressor motif. **A**: No complex was formed between Tax and the probe (**lane 2**). A complex (C1) was formed, however, when ZEB and Tax protein were added (**lane 3**). This complex was specific for the ZEB site, since a 100-fold excess of unlabeled ZEB binding oligonucleotides provided efficient competition (**lane 4**) and a negative control inhibits this complex (**lane 1**). Supershift assays confirmed that the complex contained ZEB and Tax, since the anti-ZEB and anti-Tax antibodies induced a slowly-migrating complex when added to the reaction (**lanes 5** and **6**). **B**: ZEB binds on the GATA3 silencer, forming a complex C2 (**lane 1**). This interaction was confirmed by addition of anti-ZEB (**lane 2**).

defense. Recently, GATA3 has been shown to play a role in regulating Th2 differentiation [Farrar et al., 2001]. Th2 cells are important for defense against extracellular pathogens and are implicated in atopy and allergic disease. Signaling pathways, initiated by cytokines, play a dominant role in driving the differentiation of activated naïve CD4+ T-cells into either phenotype. In response to chronic antigenic stimulation, in vivo, progressive polarization of the cytokine response ultimately leads to the commitment of Th cells to mutually exclusive Th phenotypes, which are thought to be maintained independently of extrinsic factors. GATA3 levels are low during the two phases of *TCR* gene rearrangement, but are high in the fraction of rapidly proliferating cells that insulates these two periods of TCR rearrangement. GATA3 is detected in naïve CD4+ T cells and expression levels increase substantially during Th2 differentiation [Zhang et al., 1997; Zheng and Flavell, 1997]. GATA3 plays a dual role in the in vivo differentiation of naïve Th cells into Th2 cells, by both repressing Th1 and inducing Th2 differentiation. Furthermore, various lines of evidence indicate that enforced expression of GATA3 may enhance Th2 memory cell formation. The cell fate decision of activated CD4+ cells may be regulated by GATA3, by reducing activation-induced cell death in favor of Th2 memory cell formation.

An important aspect to be considered is that transformed cells isolated from ATL patients do not express viral proteins and whether continued expression of Tax is required for transformation remains controversial [Gatza et al., 2003]. The chromosomal aberrations and genomic instability play a major role in the development and progression of multistep tumor. However, Tax functions provide the basis for HTLV-I mediated cellular transformation, in which the suppression of DNA repair pathways

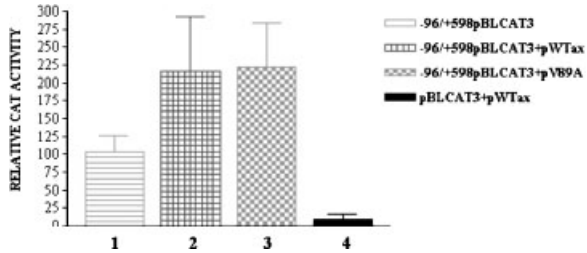

Fig. 3. Tax modestly increases the activity of the GATA3 minimal promoter. The GATA3 promoter reporter gene (−96/+598pBLCAT3) was transiently co-transfected in Jurkat cells with a CMV-based expression vector containing the coding sequence for wild-type HTLV-I Tax protein (pWTax) or Tax-derived mutant CMV-V89A (pV89A). CAT activities were detected and quantified using a CAT ELISA assay and normalized by protein level. The mean of activity for the empty plasmid, pBLCAT3, was set to 1.0 and the relative activities are presented. CAT activity of the GATA3 minimal promoter (**lane 1**). Tax protein promotes a slight increase (twofold) in the transcriptional activity of the GATA3 minimal promoter (**lane 2**), and a 2.1-fold increase (**lane 3**) was obtained with CMV-V89A Tax mutant. No relevant CAT activity was detected with the empty pBLCAT3 and pWTax (**lane 4**). The indicated values represent the means and standard deviations of three independent transfections performed in triplicate.

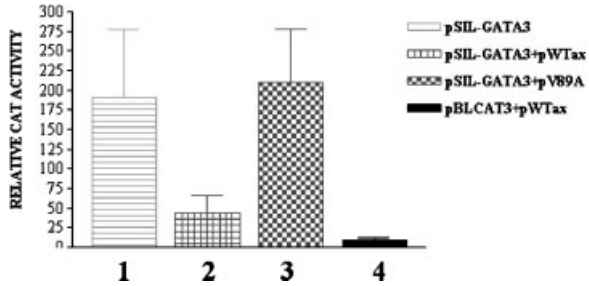

Fig. 4. Tax represses the GATA3 promoter activity in the presence of the silencer region. Jurkat cells were co-transfected with the GATA3 promoter repressor vector, pSIL-GATA3, and the vector containing the coding sequence for wild-type HTLV-I Tax protein (pWTax) or Tax-derived mutant CMV-V89A (pV89A). CAT activities were detected and quantified using a CAT ELISA assay and normalized by protein level. The mean of activity for the empty plasmid, pBLCAT3, was set to 1.0 and the relative activities are presented. As expected, an activity of the silencer in T-cells was observed (**lane 1**). pWTax vector reduces the CAT activity of the pSIL-GATA3 vector by 4.9-fold (**lane 2**). This effect was not observed with the V89A mutant (**lane 3**). No CAT activity was detected with the empty pBLCAT3 and pWTax (**lane 4**). The indicated values represent the means and standard deviations of three independent transfections in triplicate.

and the deregulation of the cell cycle lead to the gradual accumulation of mutations over time and in rare instances to the development of ATL. Recent reports described decreased expression of GATA transcription factors in esophageal carcinoma cells [Shiga et al., 1993; Lin et al., 2000], breast carcinoma [Hoch et al., 1999], and gastric carcinoma cell lines [Bai et al., 2000]. Down-regulation of GATA3 was correlated with HPV-mediated immortalization in vitro and advanced (pre) malignant cervical disease [Steenbergen et al., 2002]. Additional data derived from a study with a GATA3 mutant, which renders a dominant-negative form of GATA3, showed a polyclonal expansion of peripheral T-cells, suggesting that GATA3 plays a general role in maintaining the homeostatic balance of this cell lineage [Yamagata et al., 2000]. However, whether GATA3 down-regulation is involved in ATL pathophysiology, remains to be examined.

Our study indicates that the Tax mutation in residue 89, the amino acid that participates in the binding of CBP/p300, abolishes the Tax effect of ZEB on the GATA3 silencer, suggesting that such an effect might occur via the CBP/p300 pathway. Such results lead us to theorize that Tax could bind to ZEB through the residue 89. ZEB proteins were recently demonstrated to regulate TGFβ/BMP signaling through differential recruitment of co-activators (p300 and P/CAF). ZEB protein binds both p300 and P/CAF [Postigo et al., 2003]. The role of Tax in transactivation may extend beyond the recruitment of CBP/p300-Tax-CREB complex on the HTLV I 21-bp repeat [Harrod et al., 1998].

Finally, the presence of GATA3 in cells of the central nervous system has already been reported by Labastie et al. [1994]. The expression of this transcription factor and its regulation by the Tax protein in neural tissue deserves further investigation, due to the close association between HAM/TSP and HTLV-I.

In conclusion, the present study identified an interaction between Tax and *GATA3* gene and a role of Tax in the negative regulation of this gene expression probably through its interaction with the repressor ZEB. The CBP/p300 pathway is a potential candidate for mediating this interaction.

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