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HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 TAT ENHANCES INTERLEUKIN-2 PROMOTER ACTIVITY THROUGH SYNERGISM WITH PHORBOL ESTER AND CALCIUM-MEDIATED ACTIVATION OF THE NF-AT CIS-REGULATORY MOTIF

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Interference with T cell activation signals by Human immunodeficiency virus (HIV) gene products is suggested to contribute to the impairment of immune functions observed in AIDS. Interleukin-2 (IL-2) and HIV share common stimulatory signals triggered during T cell activation. The role of HIV tat, which is the main enhancing factor for viral LTR, in the regulation of IL-2 gene transcription has been studied following transient expression of the tat gene in phorbol ester and calcium ionophore-activated Jurkat cells transfected with IL-2 promoter-chloramphenicol acetyltransferase reporter constructs. We observed that tat increased the IL-2 promoter transcriptional activity in response to phorbol ester and ionomycin. This tat-dependent synergism mapped to the (-279 to -263 bp) NFAT motif of the IL-2 enhancer, which was sufficient to be transactivated by tat. Our data suggest that tat links T cell activating signals to the shared IL-2 and HIV regulation. This may play a role in the early phase of HIV infection.

Human immunodeficiency virus type-1 (HIV-1) interferes with several functions of the immune system. One of the suggested mechanisms is the influence of some products encoded by the viral genome on the

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molecular processes of lymphocyte activation. The HIV-1-encoded tat protein is essential for viral replication and gene expression (1). The target sequence for tat is a RNA stem-loop structure, termed TAR located at the 5' end of all HIV-1 transcripts (1). Tat cooperates with cellular proteins which participate to the transcriptional machinery, suggesting a more general role of tat in the regulation of transcription. Indeed, tat activates the expression of several cellular genes containing or not TAR elements (e.g. TGFβ, TNFβ and IL6) (2-4). As the above cytokines control immune cell functions, impairment of their regulation by tat may play a role in the pathogenesis of AIDS. In this regard, the additional involvement of IL-2 has been suggested. IL-2 is the major growth factor for T cells and plays a central role in T cell differentiation and functional activation following the interaction with antigen. Conflicting observations describe the regulation of IL-2 by tat. It has been reported that tat inhibits the antigen-induced, but not the PHA-induced, lymphocyte proliferation (5). Tat has also been described to result in decreased IL-2mRNA levels and either reduced or unchanged or increased IL-2 production, following PHA-mediated activation of T cells (6-8).

IL-2 gene transcription is activated by a broad range of signals raised by antigen-mediated T cell receptor activation or mitogen (e.g. PHA) stimulation in combination with co-stimulatory signals (9). Two of these signalling pathways involve Ras-protein kinase C and calcium-induced calcineurin, which are specifically activated by phorbol ester and calcium ionophore (9). One of the major targets of both signalling pathawys is represented by the composite NFAT motif located in the IL-2 promoter which binds the jun-fos complex together with two NFATp transcription factors related to the Rel oncogene (10). In this paper we describe that tat protein enhances the IL-2 promoter transcriptional activity through synergism with phorbol ester and calcium-mediated activation of the NFAT motif.

Materials and Methods

Plasmids: Recombinant plasmids shown in Fig. 1 are as previously described (11, 12). In pIL-2(-76)-NFAT-CAT three copies of the IL-2-NFAT-motif were inserted into pIL-2(-76)-CAT.In pCMV-tat the coding seguence of tat protein was inserted into BamHI and SalI site of pCMV derived from pTZ18R (Pharmacia, Uppsala, Sweden). The CAT gene is driven by the retinoic acid-responsive element, the human cytomegalovirus (CMV) and the SV40 early promoters and the HIV-1 LTR

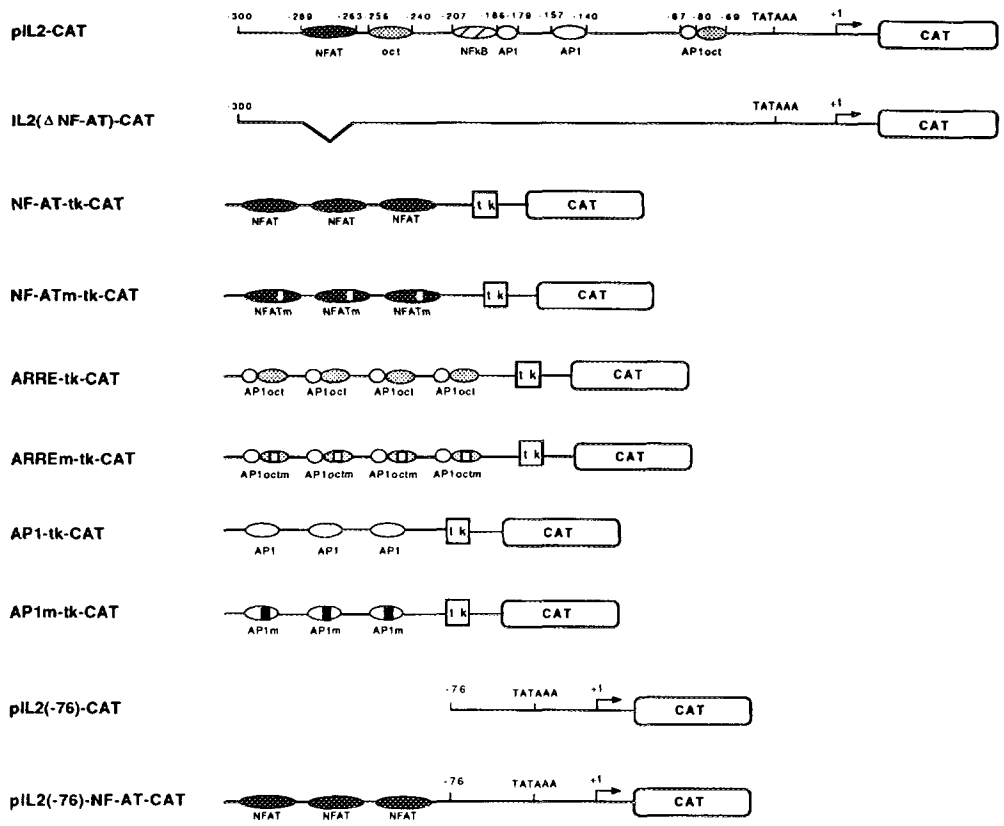

Fig. 1.
Schematic representation of reporter vectors containing IL-2 cis-regulatory sequence.

in TREp2-CAT, CMV-CAT, pSV2-CAT and TAR-1-CAT, respectively (13,14). The plasmid pCH110 contains the lacZ gene, coding for β-galactosidase (Pharmacia).

Cell culture and DNA transfection: The Jurkat T cell line was cultured in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics and transfected by the DEAE-Dextran method as described (11). All cells were cotransfected with pCH110 as an internal control for transfection efficiency. Twenty-four hours following transfection, cells were treated with 30ng/ml of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) and 2μg/ml of ionomicyn or 0.1 μM retinoic acid. After further 24h cells were harvested and protein extracts were prepared for CAT- and β-galactosidase-assays, as described (11).

Results and Discussion

To study the ability of tat to influence the transcriptional activity of the IL-2 promoter, we co-transfected an expression vector encoding the

tat gene under the control of the CMV promoter (pCMV-tat) together with the IL-2 promoter-CAT reporter (pIL-2-CAT) into Jurkat cells. A dose-dependent synergism of tat with TPA/ionomycin-mediated activation of IL-2-CAT activity was observed (up to a 5-fold enhancement compared to the empty pCMV vector) (Fig. 2A). Tat was not able to trans-activate the IL-2 enhancer in the absence of co-stimulation with TPA and ionomycin (not shown). Furthermore, tat was not able to influence the CMV (CMV-CAT) and the SV40 early promoter (pSV2CAT) and the retinoic acid-responsive element (TREp2-CAT) (Fig. 2B), suggesting that the tat-mediated enhancement of transcription is specific for the IL-2 promoter.

The IL-2 enhancer is regulated by several transcription factors which are activated by signals generated from the interaction of the T Cell Receptor with antigen or following mitogenic lectin stimulation in combination with co-stimulatory signals (e.g. CD28, other cytokines) which trigger a variety of intracellular pathways (9). These nuclear transcription factors bind to a number of cis-elements which cooperate with each other to trigger gene transcription (9). One of the strongest cis-elements is represented by the NF-AT motif located at position -279 bp to -263 bp of the 5' end of the IL-2 gene which is enhanced by activation of Ras-protein Kinase C and calcium-dependent calcineurin pathways (9). Cooperation of NF-AT with other cis-elements in the IL-2 enhancer has been reported to be required for both positive and negative gene regulation (9,11). Indeed, deletion of the NF-AT motif almost abrogated both the TPA and calcium ionophore-induced activation and the tat-mediated superinduction of the IL-2 promoter (Fig. 2B). This suggest that the NF-AT motif is essential for the activity of the IL-2 enhancer in response to TPA and calcium ionophore and might be required for the tat-mediated transactivation. To test whether the NF-AT motif was responsive to tat, we studied the ability of tat to influence the TPA/ionomycin-activated transcription from either the herpes simplex thymidine kinase (tk) or the (-76 bp)IL-2 minimal promoters driving CAT gene directed by concatemers of the NF-AT motif in comparison with multimers of other IL-2 cis-elements (Fig. 3). Fig. 3 shows that tat enhanced the TPA/ionomycin-induced transcriptional activity of NF-AT by 2 to 5-fold compared to the activation induced in the absence of tat. Mutation of the NF-AT motif, which abrogates the binding ability of nuclear factors (11), abolished both the TPA and calcium ionophore-induced activation and tat-mediated superinduction (Fig. 3). In contrast tat did not influence significantly the TPA/ionomycin-induced activation of both the (-140

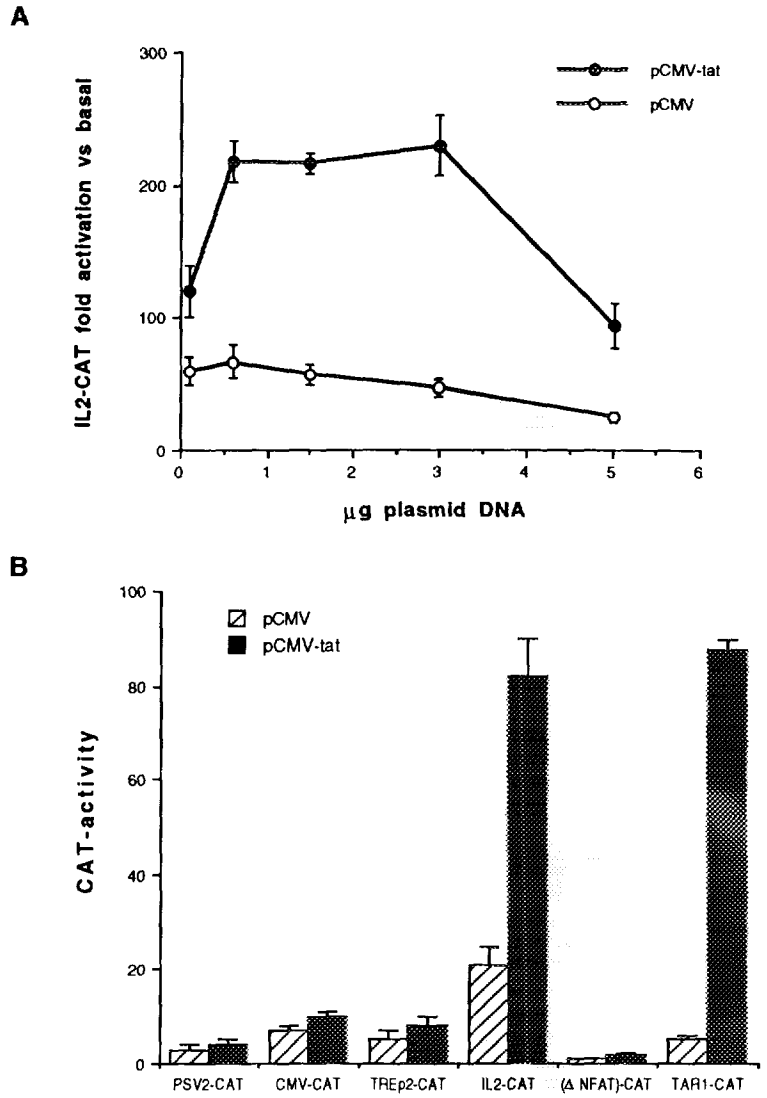

Fig. 2.
A) Dose-dependent effect of tat on TPA/ionomycin-induced activation of IL2 promoter.
Jurkat cells were co-transfected with increasing amounts of pCMV or pCMV-tat in the presence of 5 µg of pIL2-CAT. After 24 h, cells were treated with TPA/ionomycin. Results are expressed as the average (± SE) fold increase of the CAT activity observed in TPA/ionomycin treated relative to untreated (basal). Basal CAT activity, expressed as pmol/h/mg protein, ranged between 45±8 to 55±9 and 50±6 to 58±8 in the absence or in the presence of tat, respectively.
B) Effect of tat (following transfection of 1.5 µg of pCMV-CAT) on pSV2-CAT , CMV-CAT, and TAR1-CAT or retinoic acid-induced TREp2-CAT, or TPA/ionomycin-induced pIL2-CAT and (ΔNF-AT)-CAT activity. CAT activity is expressed as percent transacetylation. Background CAT activity was 1 ± 0.2%.

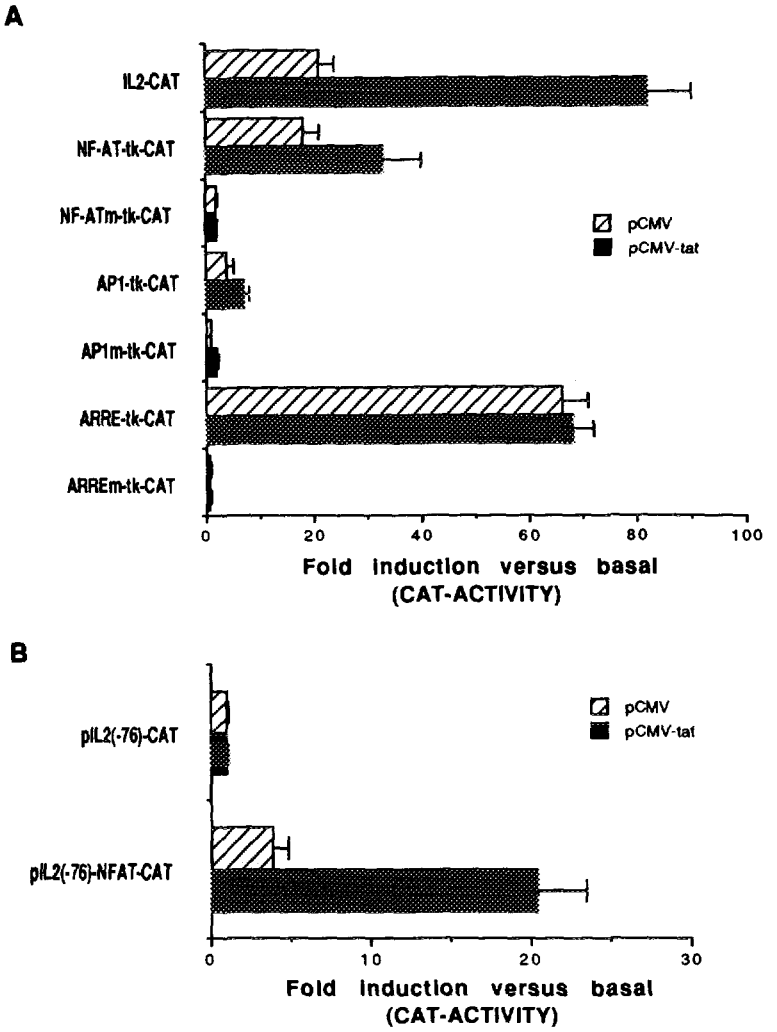

Fig. 3.
Effect of tat (1.5 µg of pCMV-CAT) on the TPA/ionomycin-induced CAT activity driven by the different IL2 cis-regulatory elements through tk (A) and IL2 minimal promoters (B), described in Fig. 1. Transfection, treatments, conditions and representation of results are described in Fig. 2A. Basal values were similar to background CAT activities and not different between the various reporter vectors.

to -150 bp) AP-1 and the (-96 to -66 bp)-ARRE motifs of the IL-2 enhancer (Fig. 3). The (-210 to -192 bp)-NF-kB binding site was not enhanced by TPA and ionomycin (11). These data suggest that the NFAT motif of the IL-2 enhancer is transactivated by tat in response to TPA and calcium ionophore.

We have described in this paper that HIV.1 tat upregulates the transcriptional response of the IL-2 enhancer to phorbol esters and

intracellular calcium increase. During the preparation of this manuscript, Westendorp et al. reported the synergism of tat with TPA and PHA in enhancing the IL-2 promoter (8). Our data confirm these observations using calcium ionophore as IL-2 inducer. Westendorp et al. mapped the tat responsive element to the -205 to -195 bp NFkB motif (8). The tat-induced activation of NF-kB factors has also been described to be involved in the TAR-independent regulation of HIV-1 LTR in T cells and of the IL-6 promoter (4,15). Furthermore, TNFβ regulation by tat requires a TAR-like structure and NF-kB (3). However, although the NF-kB motif appeared to be required for tat-mediated transactivation, it was not sufficient to respond to tat when isolated from the IL-2 enhancer context (8). This suggests the presence of additional tat-responsive cis-elements. We have reported in this paper that, in addition to the NF-kB motif previously described, the NF-AT motif is sufficient to confer to the IL-2 minimal promoter the ability to be synergistically activated by tat and TPA and calcium-induced intracellular signals. The NF-AT binding trans-acting factors include, besides jun and fos, two DNA-binding proteins harboring Rel/NF-kB-homology domains (10). Furthermore, NF-AT factors can bind to certain NF-kB sites (16). This suggests that members of the NF-kB family may be involved in the NF-AT complex. It remains therefore to be elucidated whether the tat-mediated transactivation of the NF-AT motif we have described, may involve NF-kB-like factors which participate to the NF-AT DNA-binding activity.

T cell activation is required for HIV replication and expression and further virus propagation (14). T cell activation leads to IL-2 gene stimulation which amplifies the pool of lymphocytes which can be infected by the virus. For this purpose, the IL-2 promoter and the HIV-1 LTR share stimulatory signals that trigger similar cis-elements binding common trans-acting factors (e.g. NF-AT, AP-1, NF-kB) (17). We propose that tat protein represents a further link of T cell activation to the shared IL-2 and HIV regulation. This suggests that the virus itself, through its tat product, may contribute to the T cell activation process required for virus spread and may explain the follicular hyperplasia and high proportion of activated $CD4^+$ T cells in lymph nodes during early HIV infection (18). However our observations and those reported by others (6-8) suggest the presence of complex mechanisms by which tat influences IL-2 gene expression, depending on the variety of activating signals and tat expression conditions. Indeed, at least two cis-regulatory elements appear to be responsive to tat for transcriptional regulation of IL-2 (NF-AT and NF-kB): these cis-elements may be selected by different signals. Prolonged expression of tat instead,

results in downregulation of IL-2 gene expression (6,8). The complexity of IL-2 gene regulation by tat may result in an overall dysregulation of this cytokine which could play a crucial role in the pathogenesis and evolution of AIDS.

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