JOURNAL OF VIROLOGY, July 1994, p. 4177–4185
0022-538X/94/$04.00+0

Vol. 68, No. 7

Human Immunodeficiency Virus Type 1 Tat Upregulates Interleukin-2 Secretion in Activated T Cells

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Received 10 June 1993/Accepted 6 April 1994

Dysregulation of cytokines secreted by T cells may play an important role in the pathogenesis of AIDS. To investigate the effects of human immunodeficiency virus type 1 (HIV-1) Tat on interleukin-2 (IL-2) expression, we used IL-2 promoter-chloramphenicol acetyltransferase constructs and IL-2-secreting Jurkat T cells as a model system. Transient expression of HIV-1 Tat induced a five- to eightfold increase in IL-2 promoter activity in Jurkat T cells stimulated with phytohemagglutinin and phorbol myristate acetate. IL-2 secretion was increased more than twofold in both Jurkat T cells and primary T cells stimulated by extracellular HIV-1 Tat protein. Analysis of mRNA suggested that Tat exerts its effect on IL-2 primarily at the transcriptional level. The NF-κB site at positions −206 to −195 of the IL-2 promoter was required but not sufficient for the Tat effect. The Tat-mediated increase in IL-2 promoter activity could selectively be blocked by antisense *tat* or—unlike the analogous effect of human T-cell lymphotropic virus type 1 Tax—by cyclosporin A. The observed increase in IL-2 levels might facilitate virus spread from or to T cells. Furthermore, it might contribute to the hypergammaglobulinemia or, together with other cytokines found to be dysregulated, the T-helper cell dysfunctions observed in AIDS patients.

Transactivation of human immunodeficiency virus type 1 (HIV-1) gene expression by HIV-1 Tat nuclear protein has previously been described in detail (10, 12). Tat binds to a pyrimidine bulge in the stem-loop of the transactivation response (TAR) RNA structure found at the 5′ end of all HIV-1 mRNAs. Furthermore, cellular proteins and transcription factors are required for efficient gene expression from the HIV-1 long terminal repeat (LTR) and interact with Tat (21, 49). Recent data suggest that Tat acts as a processivity factor and promotes RNA transcript elongation (16), although augmentation of transcript initiation has also been suggested (25). According to the model of Cullen (11), Tat has no direct effect on the processivity of transcription complexes that initiate at the HIV-1 LTR. Instead, it may simply activate initiation of transcription from an HIV-1 LTR promoter that is intrinsically elongation competent. Thus, activation of transcript initiation may be masked as enhanced elongation.

Recent evidence suggests that Tat may mediate additional activities regarding cell growth and function. Thus, Tat was found to stimulate growth of Kaposi's sarcoma cells (14, 15) and caused Kaposi's sarcoma-like dermal lesions in some *tat*-transgenic mice (62). Moreover, Tat was reported to suppress antigen-induced but not phytohemagglutinin (PHA)-induced proliferation of T cells (61). The *tax* gene product of human T-cell lymphotropic virus type I (HTLV-I) is a functional analog of Tat and transactivates viral gene expression (17). In addition, Tax activates cellular gene expression (63), including expression of genes like the interleukin-2 (IL-2), the transforming growth factor β (TGF-β), and the IL-2 receptor genes (9, 31, 53). Recent studies suggest that Tat, like Tax, also transactivates cellular genes, e.g., genes of the cytokines TGF-β (36), tumor necrosis factor (TNF) (4), and IL-6. Repression of gene expression by Tat has also been demonstrated, for example, for the major histocompatibility complex class I gene (26) and the manganese superoxide dismutase gene (18). Tat is secreted from HIV-1-infected or *tat*-transfected cells and can be immunoprecipitated from the supernatant of such cells (15, 19). Exogenous Tat is readily endocytosed and targeted to the nucleus of cells (38). Collectively, these observations suggest that Tat may activate expression of cellular genes in infected and noninfected cells and therefore contribute to the cytokine dysregulation observed in the course of an HIV-1 infection (6).

IL-2 has a pivotal role in the immune response. Originally described as a T-cell growth factor (40), IL-2 is known to have direct effects on growth and differentiation of T and B lymphocytes, natural killer cells, lymphokine-activated killer (LAK) cells, monocytes, macrophages, and oligodendrocytes (39). Therefore, dysregulation of IL-2 expression by Tat may have important consequences on the pathogenesis of HIV-1 infection. In this study, we describe the use of IL-2 promoter-chloramphenicol acetyltransferase (CAT) constructs and IL-2-secreting Jurkat T cells as a model system and report that endogenous and exogenous Tat enhance IL-2 expression in activated T cells.

MATERIALS AND METHODS

Cell lines and culture conditions. Jurkat T cells and HeLa cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum, 50 μg of gentamycin per ml, 6 mM HEPES (*N*-2′-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), and 2 mM L-glutamine at 37°C in a humidified atmosphere containing 5% carbon dioxide in air. A HeLa cell line stably expressing HIV-1 Tat (60), kindly provided by K. Valerie (Smith, Kline and French Laboratories, King of Prussia, Pa.), was used for cocultures.

Preparation of peripheral T cells. Human peripheral blood mononuclear cells were isolated from heparinized peripheral blood by Ficoll (Biochrom, Berlin, Federal Republic of Germany) density gradient centrifugation. Cells were washed twice

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with phosphate-buffered saline (PBS), and adherent cells were removed by incubation in supplemented medium for 1 h at 37°C. $CD19^+$ cells were depleted with the MACS magnetic microbead system (Miltenyi Biotech, Bergisch Gladbach, Federal Republic of Germany) according to the manufacturer's instructions. Purity was >99% as assessed by cytofluorometry with an anti-CD2 antibody (clone 39C1.5; Immunotech, Marseilles, France). Ten nanograms of phorbol myristate acetate (PMA) (Sigma, Deisenhofen, Federal Republic of Germany) per milliliter and 5 μg of PHA (Sigma) per ml were used for mitogen stimulation of the cells.

Recombinant plasmids. The *Hin*dIII fragment of pIL-2/CAT (52) containing the complete IL-2 promoter-enhancer region from positions −575 to +47 was inserted into the *Hin*dIII site of pBLCAT3 (37) in both orientations to yield pIL-2/CAT and pIL-2/CAT−, respectively. The HIV-1 expression vector pEXCMV-Tat contained the *tat* cDNA from pCV1 (1) under the control of the cytomegalovirus immediate-early promoter, the simian virus 40 splice signal sequence (positions 4710 to 4100), and the polyadenylation signal (positions 2770 to 2533). The HIV-1 LTR was cloned as a *Xho*I-*Hin*dIII fragment of the HIV-1 cDNA clone C15 into pSVIXCAT (55) to yield pHIV-1 LTR/CAT. pRK was used for expression of HTLV-I Tax, and pHTLV-I/CAT was used as a reporter construct. The plasmid containing four copies of the NF-κB binding sequence from the immunoglobulin kappa light-chain enhancer was generated by ligation of the synthesized NF-κB binding sequence 5′-CAGAGGGGACTTTCCGAGAGGC-3′ (34) into the polylinker site of pBLCAT2 (37). A construct containing five copies of the sequence 5′-GACCAAGAGG GATTTCACCTAAATC-3′ (positions −215 to −191 of the IL-2 promoter) inserted in pBLCAT2 was obtained from E. Serfling, University of Würzburg, Würzburg, Federal Republic of Germany (5xTCEd [50]). The antisense-*tat* expression construct (pAS-tat) pAR6 and the antisense-HIV-1 *gag* plasmid (pAS-gag) pAR2 (45) are pKEX derivatives (46).

Transfection experiments. Plasmid DNA was transfected into Jurkat T cells by the DEAE-dextran method as described by Gorman (22). Briefly, 1.5×10^7 cells were incubated with a total of 15 μg of plasmid DNA in 500 μg of DEAE-dextran and 20 μg of chloroquine per ml for 30 min. For stimulation, cultures were treated with 10 ng of PMA (Sigma) per ml and 5 μg of PHA (Sigma) per ml. Approximately 40 h after transfection, the clarified lysates were assayed for CAT activity (22) by using an automatic thin-layer chromatography linear analyzer (Berthold, Wildbad, Federal Republic of Germany) according to the manufacturer's instructions. All percent conversion values were corrected for differences in total protein concentration among samples by using the Bio-Rad protein assay system.

Mutations of the IL-2 promoter. Mutants with deletions of the 5′ end of the IL-2 promoter were synthesized by PCR according to standard protocols (29) with primers containing a *Hin*dIII or a *Bam*HI restriction site. The resulting PCR products were *Hin*dIII-*Bam*HI digested and cloned into pBLCAT3. An NF-κB point mutation of the IL-2 promoter was synthesized by thermal cycle fusion PCR (29). The oligonucleotide primers 5′-GCTAATGTAACAAAGATTTATT TCACCTACATCC-3′ and 5′-GGATGTAGGTGAAATAAA TCTTTGTTACATTAGC-3′ were used to introduce a $_{-204}$GGG-to-TTT base substitution within the NF-κB binding motif. The integrity of all IL-2 promoter mutants was confirmed by dideoxy sequencing.

Primer extension analysis. Primer extension analysis was based on the method described by Contente et al. (8). Briefly, poly(A)$^+$ RNA (3 μg) was isolated from Jurkat T cells transiently cotransfected with pIL-2/CAT and ptat or pIL-2/CAT alone. The RNA was incubated with 0.5 to 0.9 ng of ^{32}P-labeled oligonucleotide primer (binding close to the 5′ end of the CAT coding region) in 900 mM NaCl–150 mM HEPES (pH 7.6)–0.3 mM EDTA for 10 min at 68°C and then for 2 h at 30°C. The annealed primer was then precipitated with ethanol; resuspended in 20 μl of 50 mM Tris-HCl (pH 8.3)–75 mM KCl–3 mM $MgCl_2$–10 mM dithiothreitol–1 mM each dATP, dCTP, dGTP, and dTTP–1 U of RNasin (Serva, Heidelberg, Federal Republic of Germany) per μl–50 μg of RNase A per ml–25 mM EDTA for 30 min at 37°C; extracted with phenol-chloroform (1:1, vol/vol); ethanol precipitated; and run on a 6% denaturing polyacrylamide gel beside a DNA sequencing ladder.

Anti-HIV-1 Tat serum. Synthesis of full-length HIV-1 Tat (86 amino acids; HIV-1 strain BL16) will be described in detail elsewhere (18a). Anti-HIV-1 Tat serum against chemically synthesized Tat (sTat) was raised in rabbits. Rabbits were immunized six times with 150 μg of sTat at 3- to 4-week intervals. The specific anti-Tat activity was determined by enzyme-linked immunosorbent assay (ELISA). Briefly, 96-well Micro-Test plates (Becton-Dickinson, Oxnard, Calif.) were coated with 1 μg of sTat per ml or bovine serum albumin (BSA) as a control at 4°C overnight. Free sites on the plates were blocked with 1× PBS and 5% BSA for 1 h at room temperature. Plates were washed three times with 1× PBS–0.05% Nonidet P-40, incubated with anti-Tat or control serum for 90 min at room temperature, washed as described above, incubated with peroxidase-conjugated goat anti-rabbit antibody (Dianova, Hamburg, Federal Republic of Germany) for 90 min at room temperature, washed as described above, and developed with 0.1 M Na_2HPO_4–0.05 M citric acid–1.5 mg of *o*-phenylenediamine dihydrochloride per ml–0.03% H_2O_2 for 10 min in the dark. Tat-containing supernatants were preincubated with anti-Tat or control serum for 2 h at 37°C to neutralize HIV-1 Tat.

Northern (RNA) blotting and quantification of IL-2. Cytoplasmic RNA was isolated from Jurkat T cells by using the Nonidet P-40 lysis method described by Gough (23). Oligo(dT) cellulose chromatography (QuickPrep; Pharmacia, Uppsala, Sweden) was used to purify poly(A)$^+$ RNA. RNA was quantified by UV absorption at 260 nm. A 10-μg amount of poly(A)$^+$ RNA was electrophoresed in 1.0% agarose containing formaldehyde and morpholinepropanesulfonic acid (MOPS) and blotted onto a nylon membrane as previously described (35). For hybridization (47), probes for β-actin (7) and IL-2 (56) were labeled by the 3′-end-labeling system (Boehringer, Mannheim, Federal Republic of Germany). IL-2 was assayed by a human IL-2 ELISA (Hoffmann-LaRoche, Basel, Switzerland) according to the manufacturer's instructions.

RESULTS

Effect of HIV-1 Tat on the IL-2 promoter. To examine the effect of HIV-1 Tat on IL-2 expression at the transcriptional level, a *tat* cDNA expression vector (ptat) and the reporter gene construct pIL-2/CAT were cotransfected into Jurkat T cells and CAT expression was measured. Figure 1 shows that the activity of the IL-2 promoter in pIL-2/CAT was stimulated with PHA and PMA like the endogenous IL-2 gene. However, in the presence of *tat* expression and not in the presence of the expression vector lacking *tat* cDNA, IL-2 promoter activity was enhanced five- to eightfold. Mitogen (PHA and PMA) stimulation was required for this effect (Fig. 1, black bars). Similar results were obtained when the mouse IL-2 promoter instead

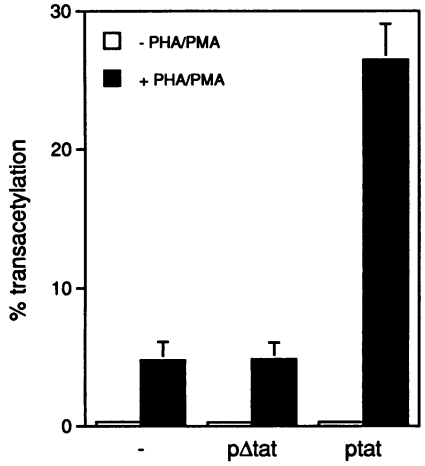

FIG. 1. Upregulation of IL-2 promoter activity in Jurkat T cells transiently transfected with *tat*. Cells were transfected with pIL-2/CAT alone (−) or cotransfected with the *tat* expression vector ptat or an equal amount of the expression vector lacking the *tat* cDNA (pΔtat). IL-2 promoter activity was measured as percent transacetylation in a CAT assay. The average values and standard deviations (error bars) for three independent transfections are shown. − PHA/PMA, no PHA and PMA stimulation; + PHA/PMA, stimulation with PHA and PMA.

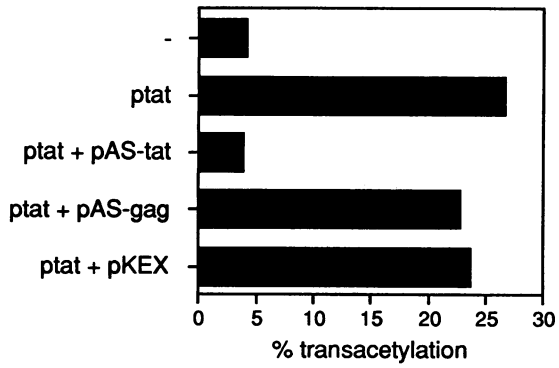

FIG. 3. Selective blocking of the effect of Tat on the IL-2 promoter. Jurkat T cells were transiently transfected with pIL-2/CAT alone (−) or cotransfected with the indicated combinations of ptat, a pKEX-based antisense RNA expression construct for *tat* (pAS-tat), *gag* (pAS-gag), or the pKEX vector itself as a control. All transfectants were stimulated with PHA and PMA as described in Materials and Methods. The IL-2 promoter activity was measured as percent transacetylation, and the values are representative of three independent transfections; variation was less than 14%.

of the human promoter was used (data not shown). Tat transactivation was promoter specific, as a β-actin promoter–CAT reporter gene construct was not influenced by cotransfection with ptat (see Fig. 4B). Furthermore, cotransfection with a reporter gene construct carrying the IL-2 promoter cassette in the opposite orientation only resulted in background CAT activity (data not shown). HIV-1 Tat might activate transcription from unusual initiation sites within the IL-2 promoter. Therefore, we performed primer extension analysis to map the RNA 5′ end. The results shown in Fig. 2 confirmed a correctly initiated, IL-2 promoter-driven transcription of the CAT gene from pIL-2/CAT in the absence and presence of Tat. Thus, HIV-1 Tat stimulates transcription from the previously described initiation site at position +1 of the IL-2 promoter (52).

Selective blocking with *tat* antisense RNA. To provide independent proof that the observed augmentation of IL-2 promoter activity was mediated by Tat, we tried to selectively block the effect. Cotransfection of equal amounts of ptat and pAS-tat, expressing antisense-*tat* RNA, selectively reduced IL-2 promoter activity to the level of activity observed with PHA and PMA stimulation in the absence of Tat (Fig. 3). Therefore, the effect of Tat on IL-2 promoter activity was blocked without affecting the basal promoter activity after mitogen stimulation. Two further controls were included to rule out the possibility of promoter interference between the cytomegalovirus immediate-early promoters of the expression vectors or an unspecific effect of antisense RNA on IL-2 promoter activity. Neither cotransfection of pKEX, the parent vector of pAS-tat, nor pKEX expressing antisense-*gag* RNA (45) had a significant effect on IL-2 promoter activity in the presence or absence of Tat. Taken together, these data support the interpretation that *tat* antisense RNA selectively blocks Tat activity.

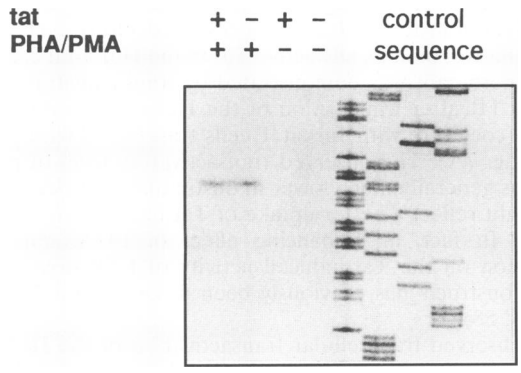

FIG. 2. Primer extension analysis of poly(A)$^+$ RNA from Jurkat T cells transiently transfected with pIL-2/CAT and ptat (tat) as indicated. Transfectants were stimulated with PHA and PMA as indicated. Equal amounts of radioactivity were loaded, and a control sequencing reaction was run beside the gel to allow determination of the fragment sizes. +, present; −, absent.

The transactivator protein of HTLV-I, termed Tax, has been shown to synergize with mitogenic stimuli like PHA and PMA in activating IL-2 gene expression (53) and to circumvent the inhibitory effects of cyclosporin A (CsA) on the IL-2 promoter (51, 53). We observed a two- to threefold CsA-resistant activation of IL-2 promoter activity in PHA-PMA-stimulated Jurkat T cells by HTLV-I Tax (Fig. 4A). These results are in accordance with previously published data (53). In contrast, CsA treatment of Jurkat T cells cotransfected with pIL-2/CAT and ptat completely blocked IL-2 promoter activity. Activity of the β-actin promoter, included as a control to rule out possible toxic effects of CsA, was not affected (Fig. 4B).

Effect of HIV-1 Tat and HTLV-I Tax on NF-κB activity. To test the assumption that transactivation of the IL-2 promoter by Tat and Tax may follow different mechanisms, we compared the effects of HIV-1 Tat and HTLV-I Tax on NF-κB activity, known to be increased by Tax (35). Thus, we tested the effects of Tax and Tat on the activity of a reporter gene construct carrying four copies of the NF-κB binding motif from the immunoglobulin kappa light-chain enhancer (κ enhancer [34]) in front of the CAT gene in transiently transfected Jurkat T cells (Fig. 5A). As expected, both mitogen stimulation and Tax resulted in a substantial increase of NF-κB activity. Further-

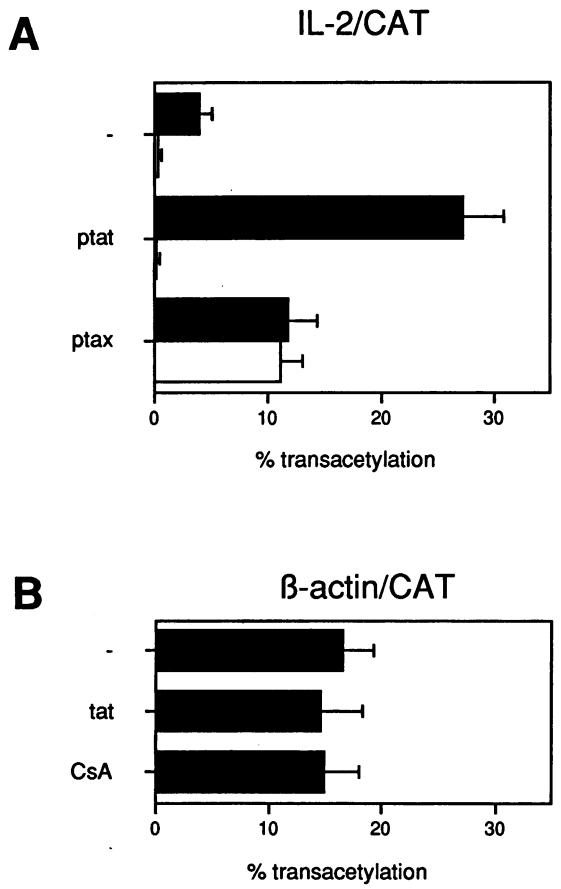

FIG. 4. (A) Comparison of the CsA sensitivities of transactivation of the IL-2 promoter by HIV-1 Tat and HTLV-1 Tax. Jurkat T cells were transiently transfected with pIL-2/CAT (−) or cotransfected with expression vectors for *tat* (ptat) or *tax* (ptax) as indicated. ■, no CsA added; □, CsA added. (B) Effect of CsA treatment and cotransfection with ptat (tat) on the activity of the β-actin promoter in pβ-actin/CAT. Cells were mitogen (PHA and PMA) stimulated as described in Materials and Methods and treated with 100 ng of CsA per ml as indicated. The promoter activity was measured as percent transacetylation. The average values and standard deviations (error bars) for three independent transfections are shown. −, cells transfected with pIL-2/CAT alone.

more, a combination of PHA and PMA and Tax had a much stronger effect on NF-κB activity than Tax or mitogen stimulation alone. However, no effect of Tat on NF-κB activity was detected. Transactivation of the IL-2 promoter by Tat requires mitogen stimulation of the cells (Fig. 1). We therefore stimulated transfected Jurkat T cells with PHA and PMA to investigate the possibility of synergistic effects of mitogen stimulation and Tat (or Tax) on NF-κB activity. As shown in Fig. 5A, Tat did not synergize with PHA and PMA to increase NF-κB activity. The NF-κB binding sequences in the immunoglobulin kappa light-chain enhancer and the IL-2 enhancer differ slightly (see Materials and Methods). Therefore, we tested a reporter gene construct carrying four copies of the NF-κB motif from the IL-2 promoter (Fig. 5B). Again, no effect of Tat on NF-κB activity was observed in unstimulated and PHA-PMA-stimulated Jurkat T cells.

To validate these results in the context of the intact IL-2 promoter, we generated an NF-κB point mutation shown previously to abolish NF-κB binding (2). Both the wild type and the mutated IL-2 promoter construct were tested with PHA-PMA-stimulated Jurkat T cells transiently cotransfected with ptat (Fig. 6, upper two bars). Interestingly, the NF-κB mutation abolished the ability of HIV-1 Tat to transactivate the IL-2 promoter. Therefore, the NF-κB site at positions −206 to −195 of the IL-2 promoter is necessary but—as judged from the multimerized NF-κB reporter constructs (Fig. 5)—not sufficient for transactivation by HIV-1 Tat.

Apart from the NF-κB motif, the IL-2 promoter contains various sites that bind proteins believed to contribute to transcriptional control, including those for NF-AT, AP-1, Oct-1/2, CD28RC, and others (20, 48). These sites within the IL-2 promoter have previously been grouped into five functional boxes (48). On the basis of this division, we constructed a series of 5′ deletion mutants and cloned them into pBLCAT3. The ability of Tat to transactivate the truncated promoter constructs was tested by transient cotransfection of Jurkat T cells as described above. As shown in Fig. 6, deletion of the 5′ region of the IL-2 promoter to position −208 did not affect Tat transactivation, though PHA and PMA stimulation was reduced (Fig. 6 legend). Therefore, neither the previously described NF-AT site (positions −286 to −257), the Oct-1/2 site (positions −256 to −242), nor any potential unknown binding motif upstream of position −208 is required for Tat transactivation of the IL-2 promoter. In contrast, further deletion of the sequence between positions −208 and −158 abolished most of the Tat transactivation. This part of the IL-2 promoter contains the AP-1 distal site (positions −185 to −177) and the NF-κB site discussed above. The results confirm the data obtained from the NF-κB point mutation (see above) and the important role of the NF-κB motif in transactivation of the IL-2 promoter by Tat. Further 5′ deletion of the IL-2 promoter resulted in loss of the residual Tat responsiveness. However, this effect was only marginally significant and transacetylation rates obtained with the respective constructs were close to background levels. In summary, the sequence between positions −208 and +51 of the IL-2 promoter is sufficient for transactivation by Tat and the NF-κB motif is a key element in this sequence.

Effect of exogenous Tat on the IL-2 promoter. Tat secreted by one cell might be taken up by neighboring cells and exert its effect on IL-2 promoter activity of the latter cells. Such mechanisms might be of particular importance in situations where infected Tat-producing T cells come into close contact with uninfected T cells, e.g., in lymphoid tissue of HIV-1-infected individuals. To test this hypothesis, we analyzed the effect of HeLa cells stably transfected with *tat* (HeLa-tat) on pIL-2/CAT-transfected Jurkat T cells in cocultures. The presence of native, biologically active Tat in the HeLa-tat cells and their supernatant was demonstrated by transactivation of the HIV-1 LTR after transfection of the HeLa cells with pLTR/CAT or coculture with Jurkat T cells transfected with pLTR/CAT (Fig. 7A). The observed transactivation level of pLTR/CAT was generally much lower in unstimulated Jurkat T cells. This might reflect a better uptake of Tat into activated Jurkat T cells. In fact, an enhancing effect of PHA and PMA stimulation on the Tat-induced activity of LTR-driven CAT vector constructs has previously been described for Jurkat T cells (54, 58).

The observed transcellular transactivation of the IL-2 promoter might be either directly mediated by Tat or indirectly mediated via a cytokine secreted from HeLa-tat cells. Therefore, we used rabbit serum raised against synthetic HIV-1 Tat to neutralize Tat activity in the supernatant of HeLa-tat cells. Normal rabbit serum was used as a control. As shown in Fig. 8, pretreatment of HeLa-tat cell supernatant with anti-Tat se-

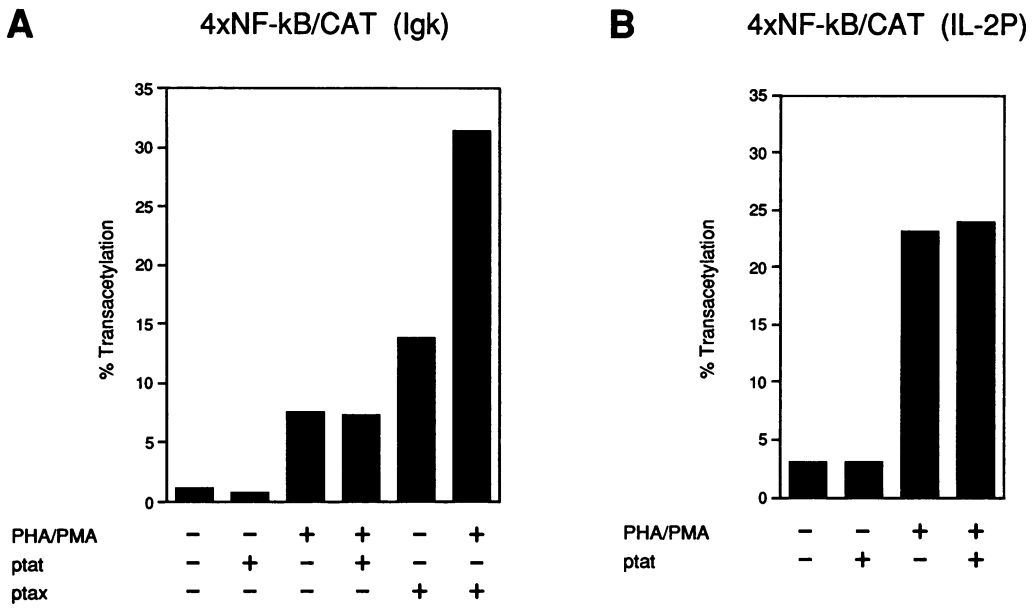

FIG. 5. Effect of HIV-1 Tat and HTLV-I Tax on NF-κB activity. Jurkat T cells were transiently cotransfected with a reporter gene construct carrying four copies of the NF-κB binding sequence (4×NF-κB) from the immunoglobulin kappa light chain enhancer (Igk) (A) or the IL-2 promoter (IL-2P) (B) in front of the CAT gene and expression vectors for *tat* (ptat) or *tax* (ptax) as indicated. Cells were mitogen (PHA and PMA) stimulated as shown. The NF-κB activity was measured as percent transacetylation. Values are representative of three independent transfections; variation was less than 18%. +, present; −, absent.

rum, but not with normal rabbit serum, blocked transactivation of the IL-2 promoter in transiently transfected Jurkat T cells. This blocking effect was concentration dependent and was no longer observed with high dilutions of anti-Tat serum. The results shown in Fig. 7B and C and Fig. 8 demonstrate the increase of IL-2 promoter activity by exogenous Tat. Both HeLa-tat cells (Fig. 7C) and their Tat-containing supernatant (Fig. 7B) caused a two- to threefold increase in PHA-PMA-stimulated IL-2 promoter activity in Jurkat T cells. These data demonstrate that HIV-1 Tat may regulate expression of genes in noninfected cells.

Effect of extracellular Tat on the endogenous IL-2 gene. An artificial IL-2 promoter–CAT fusion construct differs from the

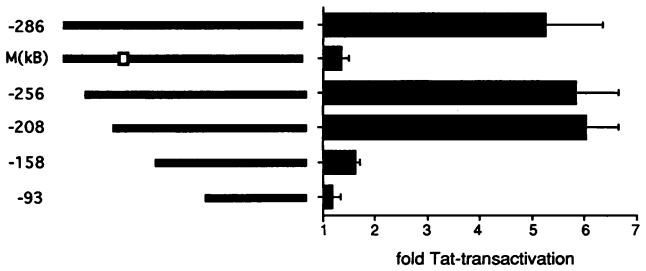

FIG. 6. Transactivation of 5′ deletion mutants (the position of the 5′ end of the truncated promoter sequences is indicated; in all mutants, the 3′ end was at position +47) and an NF-κB mutant [M(kB)] with mutations of the IL-2 promoter by HIV-1 Tat. The fold transactivation of the promoter-CAT constructs by cotransfection with ptat is shown. The average values and standard deviations (error bars) for three independent transfections are shown. Fold activation and average transacetylation rates (in parentheses) after PHA and PMA stimulation were as follows: −286, 32 (7.2%); −256, 12 (3.4%); −208, 6 (2.7%); −157, 2.5 (1.3%); −93, 1.8 (0.9%); M(kB), 17 (4.4%).

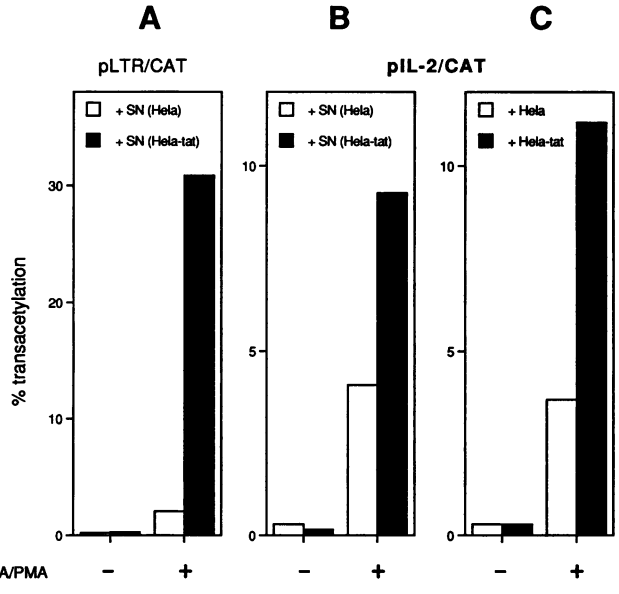

FIG. 7. Upregulation of IL-2 promoter activity by extracellular Tat. (A) HIV-1 LTR promoter activity measured as CAT activity in Jurkat T cells transiently transfected with pLTR/CAT and cultured in supernatant of HeLa-tat or HeLa cells as a control. (B and C) IL-2 promoter activity measured as CAT activity in Jurkat T cells transiently transfected with pIL-2/CAT and cocultured with a HeLa cell line stably transfected with *tat* (Hela-tat) or the wild-type HeLa cell line (Hela) (C) or cultured in supernatants (SN) of the latter cells (B). Values are representative of three independent transfections; variation was less than 16%. The supernatants of HeLa-tat and wild-type HeLa cells did not influence proliferation of Jurkat T cells in the presence or absence of mitogens (data not shown). +, present; −, absent.

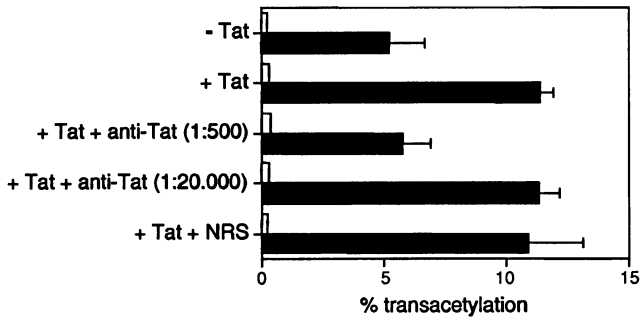

FIG. 8. Blocking of the effect of extracellular Tat by anti-Tat serum. IL-2 promoter activity was measured as CAT activity in Jurkat T cells transiently transfected with pIL-2/CAT and cultured in supernatant of HeLa-tat (+ Tat) or HeLa (− Tat) cells as a control. Cells were mitogen (PHA and PMA) stimulated (■) or unstimulated (□) as shown. anti-Tat, treatment of the HeLa-tat supernatant with anti-Tat serum, diluted 1:500 or 1:20,000; NRS, normal rabbit serum used as a control. The average values and standard deviations (error bars) for three independent experiments are shown.

endogenous IL-2 gene with respect to the flanking DNA sequences, the RNA transcript, and possibly the chromatin structure. This difference might result in a different regulation and expression pattern. Therefore, we also tested expression of the endogenous IL-2 gene in Jurkat T cells cultured in Tat-containing supernatant from HeLa-tat cells. Figure 9 shows that exposure to extracellular, native Tat resulted in an approximately twofold increase of IL-2 mRNA levels only 4 h after mitogen stimulation, whereas the level of β-actin mRNA used as a control was unaffected. IL-2 mRNA levels 20 h after stimulation were comparable in the absence and presence of Tat.

To investigate whether the effect of Tat on secretion of IL-2 could also be observed, we measured IL-2 in the supernatant of Jurkat T cells and purified peripheral T cells cultured in the presence or absence of exogenous Tat and stimulated with PHA and PMA for 15 h. Figure 10A shows that IL-2 in the supernatant of Jurkat T cells was increased more than twofold when Tat was present in the culture medium.

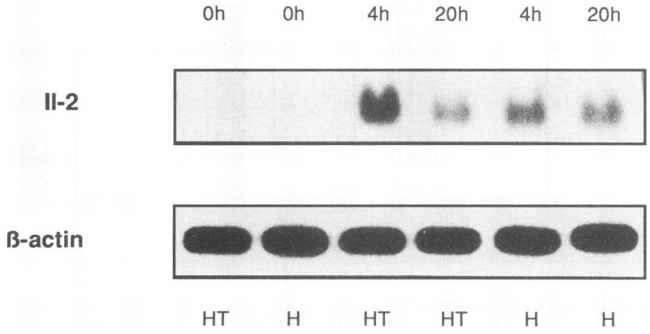

FIG. 9. Detection of IL-2 mRNA in Jurkat T cells. Northern blotting of poly(A)$^+$ mRNA from Jurkat T cells cultured for 12 h in Tat-containing supernatant of HeLa-tat cells (HT) or of wild-type HeLa cells (H) and stimulated with PHA and PMA for the indicated time was performed by using probes specific for IL-2 or β-actin as a control. Results are representative of three independent experiments. The average fold induction (± standard deviation) of IL-2 mRNA by Tat as measured by densitometry was 2.16 (± 0.44) at 4 h and 1.18 (± 0.37) at 20 h.

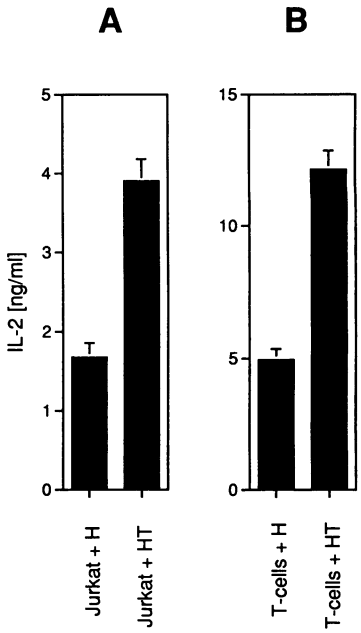

FIG. 10. Upregulation of IL-2 secretion from activated Jurkat (A) and peripheral (B) T cells by exogenous Tat. Jurkat T cells or purified peripheral T cells were cultured for 12 h in Tat-containing supernatant of HeLa-tat cells (HT) or supernatant of wild-type HeLa cells (H) as a control. IL-2 secretion was measured 15 h after PHA and PMA stimulation by using an IL-2 ELISA. The average values and standard deviations (error bars) for three independent experiments are shown.

Normal T cells and malignant Jurkat T cells differ in growth control and requirements for growth factors like IL-2 and IL-4. Therefore, we tested whether Tat also influences IL-2 secretion from activated normal peripheral T cells. Figure 10B demonstrates the same significant enhancement of IL-2 secretion by Tat in activated normal peripheral T cells as observed in Jurkat T cells.

DISCUSSION

The data presented in this paper show that HIV-1 Tat upregulates IL-2 gene expression and IL-2 secretion in activated Jurkat T cells and in freshly isolated activated peripheral T cells. The effects of Tat on promoter activity, mRNA expression, and IL-2 secretion suggested that IL-2 upregulation was primarily caused at the transcriptional level. Our experiments addressed the short-term effects of HIV-1 Tat on T cells. The long-term effects of Tat on T cells, however, might be different. Thus, in accordance with previous observations (43), we also found decreased IL-2 expression in stable *tat*-transfected mitogen-activated Jurkat T cells (data not shown). Therefore, prolonged high-level expression of Tat in T cells might result in suppression rather than stimulation of IL-2 expression. This suggests that the effects of Tat might depend on the duration of exposure of T cells to Tat. Upregulation of IL-2 expression might therefore only be transient in stable Tat transfectants or in T cells of HIV-1-infected individuals. This assumption is supported by our observation that IL-2 mRNA levels were strongly increased by Tat 4 h after mitogen stimulation and reached normal levels 16 h later. The differential effects of Tat on IL-2 secretion of T cells, upregulation upon short-term exposure and downregulation upon long-term

exposure, might also make detection of IL-2 levels in serum of HIV-1-infected individuals difficult (6).

The mechanism of the Tat effect on IL-2 expression remains partly unclear. Sequence comparison of the IL-2 RNA and TAR, the Tat-binding site of the HIV-1 LTR RNA, revealed no binding site for Tat on IL-2 RNA. Recently, the presence of stem-loop structures containing the CUGGGA sequence downstream of the transcriptional start site of the TNF-β promoter has been suggested to play a role in Tat-induced TNF-β activation (4). Similar sequences forming TAR-like stem-loops have been identified downstream of the RNA initiation site of the JC virus promoter (5, 44). However, no such sequences are present at the 5′ end of IL-2 RNA. Therefore, it is likely that transactivation of IL-2 expression by Tat represents a new TAR-independent mechanism of transcriptional activation. However, the binding of Tat to a stem-loop structure, homologous to but distinct from TAR, at the 5′ end of IL-2 RNA which might—as in the case of the HIV-1 LTR—tether Tat to the promoter cannot completely be ruled out. Comparison of HIV-1 Tat and the transactivator of a related human retrovirus, HTLV-I Tax, also known to synergize with mitogens to stimulate IL-2 expression (53), revealed clear differences but also similarities in transactivation of the IL-2 promoter. The effect of Tat was completely blocked with relatively low concentrations of the immunosuppressant CsA. Tax, however, was shown to circumvent the effects of CsA (Fig. 4) (51, 53). Moreover, Tax, but not Tat, could transactivate a multimerized NF-κB binding motif. However, both transactivators synergized with PHA and PMA stimulation of cells in transactivation of IL-2 gene expression. Though the NF-κB binding sequence was not sufficient for transactivation by Tat, it was still required to confer Tat responsiveness to the IL-2 promoter. Interestingly, the enhancer element of the HIV-1 LTR, composed of two NF-κB motifs (3), and Tat are required for TAR-independent activation of HIV-1 gene expression in PMA-treated Jurkat T cells (24) and glial cells (57). Thus, Tat may be capable of interacting directly with certain κB binding proteins present in activated T cells and glial cells and can associate with promoters independently of TAR (28). Tat transactivation of the IL-2 promoter is probably an important example of such a mechanism. Our results suggest involvement of the NF-κB element and at least one additional element downstream of position −208 (Fig. 6) in transactivation of the IL-2 promoter by Tat. Possible elements downstream of this position include NF-AT, AP-1, OAP, Oct-1/2, and CD28RC. Of these, NF-AT-1, AP-1 (41), and OAP (59) have been described to be CsA sensitive. The sensitivity of these elements would provide an explanation for blocking of the Tat but not the Tax effect by the immunosuppressant. Further mutational analysis of the IL-2 promoter is necessary to identify additional requirements of Tat transactivation and elements interacting with Tat. Thus, an influence of Tat on the activity of specific transcription factors or regulatory elements has recently been described for the murine cytomegalovirus major immediate-early promoter (32). Furthermore, a direct (27) and functional (30) interaction of Tat and the transcription factor Sp1 has been demonstrated for the HIV-1 LTR.

Intracellular expression of Tat in transfected Jurkat T cells may not correctly represent the pathological situation for AIDS. Consequently, its biological significance may be difficult to interpret. Therefore, it is particularly significant that the results presented here are not restricted to intracellular Tat. Extracellular Tat, secreted by Tat-producing cells, was taken up by neighboring T cells and transactivated IL-2 expression after mitogen stimulation. This effect could specifically be blocked with anti-Tat antibodies. In our experiments, endogenous Tat evoked a much stronger increase in IL-2 promoter activity than did exogenous Tat (a five- to eightfold versus a two- to threefold increase). This difference is most likely explained by a lower concentration of secreted (exogenous) Tat in the culture medium. Thus, exposure of cells to exogenous Tat may yield lower intracellular concentrations of Tat than does Tat expression within the cell. In accordance, concentration dependence of Tat effects has recently been demonstrated for purified recombinant Tat (15). In vivo, Tat may be concentrated in lymphoid tissue at sites where HIV-1 replication is most active during the clinically latent period of HIV-1 infection (13, 42). In addition, close contact of cells at such sites might make uptake of Tat by surrounding noninfected T cells particularly efficient. This effect may therefore exceed the effect observed with our cell cultures. In conclusion, we suggest that Tat produced and secreted by infected lymphocytes or macrophages in the lymphoid tissue is taken up by surrounding noninfected T cells and influences their IL-2 production. Ensuing fluctuations of IL-2 levels in the lymphoid microenvironment might then severely influence T-cell function in HIV-1 infection.

T-cell activation is known to be required for efficient virus propagation. Therefore, increased IL-2 levels might enhance viral replication and virus spread. In addition, increased IL-2 levels are in accordance with and might help to explain the high frequency of activated T cells and follicular hyperplasia and a high rate of viral replication observed in lymphoid tissue early in the course of HIV-1 infection (42). HIV-1 Tat-mediated dysregulation of IL-2 and possibly other inflammatory cytokines (4) might contribute to the apparent immunologic dysfunction observed in AIDS patients, or to the development of Kaposi's sarcoma (14, 15). Moreover, an increase in IL-2 levels caused by Tat might be a factor that contributes to the hypergammaglobulinemia described in connection with AIDS (33).

Further studies are necessary to clarify the physiologic role of transactivation of IL-2 expression in activated T cells in HIV-1 infection and to elucidate the molecular mechanism underlying the action of Tat on cellular gene expression.

ACKNOWLEDGMENTS

We thank E. Serfling, K. Khazaie, G. Sczakiel, M. Pawlita, and H. G. Kräusslich for vector constructs and helpful discussions; K. Valerie for providing the HeLa-tat cell line; and D. Heiss, C. Bürkle, and M. Kraft for expert technical assistance.

This work was supported by grants from the Bundesministerium für Forschung und Technologie, Bonn, Federal Republic of Germany.

REFERENCES

1. **Arya, S., and R. C. Gallo.** 1986. Three novel genes of the human T-lymphotrophic virus type III: immune reactivity of their products with sera from acquired immune deficiency syndrome patients. Proc. Natl. Acad. Sci. USA **83**:2209–2213.
2. **Baeuerle, P. E., and D. Baltimore.** 1991. The physiology of the NF-κB transcription factor, p. 423. *In* P. Cohen and J. G. Foulkes (ed.), Molecular aspects of cellular regulation 6: the hormonal control of gene transcription. Elsevier Science Publishers BV, Amsterdam.
3. **Berkhout, B., and K. T. Jeang.** 1992. Functional roles for the TATA promoter and enhancers in basal and Tat-induced expression of the human immunodeficiency virus type 1 long terminal repeat. J. Virol. **66**:139–149.
4. **Buonaguro, L., G. Barillari, H. K. Chang, C. A. Bohan, V. Kao, R. Morgan, R. C. Gallo, and B. Ensoli.** 1992. Effects of the human immunodeficiency virus type 1 Tat protein on the expression of inflammatory cytokines. J. Virol. **66**:7159–7167.
5. **Chowdhury, M., J. P. Taylor, C.-F. Chang, J. Rappaport, and K. Khalili.** 1992. Evidence that a sequence similar to TAR is impor-

tant for induction of the JC virus late promoter by human immunodeficiency virus type 1 Tat. J. Virol. **66**:7355–7361.
6. **Clerici, M., and G. M. Shearer.** 1993. A Th1 → Th2 switch is a critical step in the etiology of HIV-1 infection. Immunol. Today **14**:107–111.
7. **Cleveland, D. W., M. A. Lopata, R. J. MacDonald, N. J. Cowan, W. J. Rutter, and M. W. Kirschner.** 1980. Number and evolutionary conservation of α- and β-tubulin and cytoplasmic β- and γ-actin genes using specific cloned cDNA probes. Cell **20**:95–105.
8. **Contente, S., K. Csiszar, K. Kenyon, and R. M. Friedman.** 1993. Structure of the mouse lysyl oxidase gene. Genomics **16**:395–400.
9. **Cross, S. L., J. B. Feinberg, J. B. Wolf, N. J. Holbrook, F. Wong-Staal, and W. Leonard.** 1987. Regulation of the human interleukin-2 receptor alpha chain promoter: activation of a nonfunctional promoter by the trans-activator gene of HTLV-I. Cell **49**:47–56.
10. **Cullen, B. R.** 1986. Regulation of HIV-1 gene expression. FASEB J. **5**:2361–2368.
11. **Cullen, B. R.** 1993. Does HIV-1 Tat induce a change in viral initiation rights? Cell **73**:417–420.
12. **Dingwall, C. I., I. Ernberg, C. H. Chen, S. M. Green, S. Heaphy, J. Karn, A. S. Lowe, M. Singh, and M. A. Skinner.** 1990. HIV-1 tat protein stimulates transcription by binding to a U-rich bulge in the stem of the TAR RNA structure. EMBO J. **9**:4145–4153.
13. **Embretson, J., M. Zupancic, J. L. Ribas, A. Burke, J. Tenner-Racz, and A. T. Haase.** 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature (London) **362**:359–362.
14. **Ensoli, B., G. Barillari, S. Z. Salahuddin, R. C. Gallo, and F. Wong-Staal.** 1990. Tat protein of HIV-1 stimulates growth of cells derived from Kaposi's sarcoma lesions of AIDS patients. Nature (London) **345**:84–86.
15. **Ensoli, B., L. Buonaguro, G. Barillari, V. Fiorelli, R. Gendelman, R. A. Morgan, P. Wingfield, and R. C. Gallo.** 1993. Release, uptake, and effects of extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral transactivation. J. Virol. **67**:277–287.
16. **Feinberg, M. B., D. Baltimore, and A. D. Frankel.** 1991. The role of Tat in the human immunodeficiency virus life cycle indicates a primary effect on transcriptional elongation. Proc. Natl. Acad. Sci. USA **88**:4045–4049.
17. **Felber, B. K., H. Paskalis, C. Kleinmann-Ewing, F. Wong-Staal, and G. N. Pavlakis.** 1985. The pX protein of HTLV-1 is a transcriptional activator of its long terminal repeat. Science **229**: 675–679.
18. **Flores, S. C., J. C. Marecki, K. P. Harper, S. K. Bose, S. K. Nelson, and J. M. McCord.** 1993. Tat protein of human immunodeficiency virus type 1 represses expression of manganese superoxide dismutase in Hela cells. Proc. Natl. Acad. Sci. USA **90**:7632–7636.
18a. **Frank, R. W., et al.** Unpublished data.
19. **Frankel, A. D., and C. O. Pabo.** 1988. Cellular uptake of the tat protein from human immunodeficiency virus. Cell **55**:1189–1193.
20. **Fraser, J. D., B. A. Irving, G. R. Crabtree, and A. Weiss.** 1991. Regulation of the IL-2 gene enhancer activity by the T cell accessory molecule CD28. Science **251**:313–316.
21. **Garcia, J. A., F. A. Wu, R. Mitsuyasu, and R. B. Gaynor.** 1987. Interaction of cellular proteins involved in the transcriptional regulation of human immunodeficiency virus. EMBO J. **6**:3761–3770.
22. **Gorman, C.** 1985. High efficiency gene transfer into mammalian cells, p. 143. *In* D. M. Glover (ed.), DNA cloning, vol. II. IRL press, Washington, D.C.
23. **Gough, N. M.** 1988. Rapid and quantitative preparation of cytoplasmic RNA from small numbers of cells. Anal. Biochem. **173**:93–95.
24. **Harrich, D., J. Garcia, R. Mitsuyasu, and R. Gaynor.** 1990. TAR independent activation of the human immunodeficiency virus in phorbol ester stimulated T lymphocytes. EMBO J. **9**:4417–4423.
25. **Hauber, J., A. Perkins, E. P. Heimer, and B. R. Cullen.** 1987. Trans-activation of human immunodeficiency virus gene expression is mediated by nuclear events. Proc. Natl. Acad. Sci. USA **84**:6364–6368.
26. **Howkroft, T. K., K. Strebel, M. A. Martin, and D. S. Singer.** 1993. Repression of MHC class I gene promoter by two-exon Tat of HIV. Science **260**:1320–1322.
27. **Jeang, K.-T., R. Chun, N. H. Lin, A. Gatignol, C. G. Glabe, and H. Fan.** 1993. In vitro and in vivo binding of human immunodeficiency virus type 1 Tat protein and Sp1 transcription factor. J. Virol. **67**:6224–6233.
28. **Jones, K. A.** 1993. Tat and the HIV-1 promoter. Curr. Opin. Cell Biol. **5**:461–468.
29. **Kahn, S. M., J. Wei, C. Borner, K. O'Driscoll, and B. Weinstein.** 1990. Construction of defined deletion mutants by thermal cycled fusion: applications to protein kinase C. Technique **2**(I):27.
30. **Kamine, J., and G. Chinnadurai.** 1992. Synergistic activation of the human immunodeficiency virus type 1 promoter by the viral Tat protein and cellular transcription factor Sp1. J. Virol. **66**:3932–3936.
31. **Kim, Y.-S., J. H. Kehrl, J. Burton, C. L. Tendler, K. T. Jeang, D. Danielpour, C. Thevenin, K. Y. Kim, M. B. Sporn, and A. B. Roberts.** 1990. Transactivation of the transforming growth factor β1 (TGF-β1) gene by human T lymphotropic virus type I Tax: a potential mechanism for the increased production of TGF-β1 in adult T-cell leukemia. J. Exp. Med. **172**:121–129.
32. **Kim, Y.-S., and R. Risser.** 1993. TAR-independent transactivation of the murine cytomegalovirus major immediate-early promoter by the Tat protein. J. Virol. **67**:239–248.
33. **Lane, H. C., H. Masur, M. S. Edgar, G. Whalen, A. H. Rook, and A. S. Fauci.** 1983. Abnormalities of B-cell activation and immunodeficiency syndrome. N. Engl. J. Med. **309**:453–458.
34. **Lenardo, M. J., A. Kuang, A. Gifford, and D. Baltimore.** 1988. NF-κB protein purification from bovine spleen: nucleotide stimulation and binding site specificity. Proc. Natl. Acad. Sci. USA **85**:8825–8829.
35. **Leung, K., and G. J. Nabel.** 1988. HTLV-1 transactivator induces interleukin-2 receptor expression through an NF-kappa B-like factor. Nature (London) **333**:776–778.
36. **Lotz, M., J. Keckow, M. T. Cronin, J. A. McCutchan, I. Clark-Lewis, D. A. Carons, and W. Wachsman.** 1990. Induction of transforming growth factor β (TGFβ) by HIV-1 Tat; a noncytopathic pathway of immunodeficiency in HIV infection. FASEB J. **4**:1861.
37. **Luckow, B., and G. Schütz.** 1987. CAT constructions with multiple unique restriction sites for the functional analysis of eukaryotic promoters and regulatory elements. Nucleic Acids Res. **15**:5490.
38. **Mann, D. A., and A. D. Frankel.** 1991. Endocytosis and targeting of exogenous HIV-1 Tat protein. EMBO J. **10**:1733–1739.
39. **Minami, Y., T. Kono, T. Miyazaki, and T. Taniguchi.** 1993. The IL-2 receptor complex: its structure, function and target genes. Annu. Rev. Immunol. **11**:245–267.
40. **Morgan, D. A., F. W. Ruscetti, and R. C. Gallo.** 1976. Selective in vitro growth of T lymphocytes from normal human bone marrows. Science **193**:1007–1008.
41. **Paliogianni, F., A. Raptis, S. S. Ahuja, S. M. Najjar, and D. T. Boumpas.** 1993. Negative transcriptional regulation of human interleukin-2 gene by glucocorticoids through interference with nuclear transcription factors AP-1 and NF-AT. J. Clin. Invest. **91**:1481–1489.
42. **Pantaleo, G., C. Graziosi, H. F. Demarest, L. Butini, M. Montroli, C. H. Fox, J. M. Orenstein, D. P. Kotler, and A. S. Fauci.** 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of the disease. Nature (London) **362**: 355–358.
43. **Purvis, S. F., D. L. Georges, T. M. Williams, and M. M. Lederman.** 1992. Suppression of interleukin-2 and interleukin-2 receptor expression in Jurkat cells stably expressing the human immunodeficiency virus Tat protein. Cell. Immunol. **144**:32–42.
44. **Remenick, J., M. F. Radonovich, and J. N. Brady.** 1991. Human immunodeficiency virus Tat transactivation: induction of a tissue-specific enhancer in a nonpermissive cell line. J. Virol. **65**:5641–5646.
45. **Rittner, K., and G. Sczakiel.** 1991. Identification and analysis of antisense RNA target regions of the human immunodeficiency virus type 1. Nucleic Acids Res. **19**:1421–1426.
46. **Rittner, K., H. Stöppler, M. Pawlita, and G. Sczakiel.** 1991. Versatile eukaryotic vectors for strong and constitutive transient

and stable expression. Methods Mol. Cell. Biol. **2**:176–181.
47. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
48. **Schreiber, S. L., and G. R. Crabtree.** 1992. The mechanism of action of cyclosporin A and FK506. Immunol. Today **13**:136–142.
49. **Selby, M. J., and B. M. Peterlin.** 1990. Trans-activation by HIV-1 Tat via a heterologous RNA binding protein. Cell **62**:769–776.
50. **Serfling, E., R. Barthelmäs, I. Pfeuffer, B. Schenk, S. Zarius, R. Swoboda, F. Merkurio, and M. Karin.** 1989. Ubiquitous and lymphocyte-specific factors are involved in the induction of the mouse interleukin 2 gene in T lymphocytes. EMBO J. **8**:465.
51. **Shibuya, H., and T. Taniguchi.** 1989. Identification of multiple cis-elements and trans-acting factors involved in the induced expression of human IL-2 gene. Nucleic Acids Res. **17**:9173–9184.
52. **Siebenlist, U., D. B. Durand, P. Bressler, N. J. Holbrook, C. A. Norris, M. Kamoun, J. A. Kant, and G. R. Crabtree.** 1986. Promoter region of interleukin-2 gene undergoes chromatin structure changes and confers inducibility on chloramphenicol acetyltransferase gene during activation of T cells. Mol. Cell. Biol. **6**:3042–3049.
53. **Siekevitz, M., M. B. Feinberg, N. Holbrook, F. Wong-Staal, and W. C. Greene.** 1987. 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. Proc. Natl. Acad. Sci. USA **84**:5389–5393.
54. **Siekevitz, M., S. F. Josephs, M. Dukovich, N. Peffer, F. Wong-Staal, and W. C. Greene.** 1987. Activation of the HIV-1 LTR by T cell mitogens and the transactivator protein of HTLV-1. Science **238**:1575–1578.
55. **Sodroski, J., R. Patarca, and C. Rosen.** 1985. Location of the transacting region on the genome of human T-lymphotrophic virus type III. Science **229**:74–77.
56. **Taniguchi, T., H. Matsui, T. Fujita, C. Takaoka, N. Kashima, R. Yoshimoto, and J. Hamuro.** 1983. Structure and expression of a cloned cDNA for human interleukin-2. Nature (London) **302**:302–310.
57. **Taylor, J. P., R. Pomerantz, O. Bagasra, M. Chowdhury, J. Rappaport, K. Khalili, and S. Amini.** 1992. TAR-independent transactivation by Tat in cells derived from the CNS: a novel mechanism of HIV-1 gene regulation. EMBO J. **11**:3395–3403.
58. **Tong-Starksen, S. E., P. A. Luciw, and B. M. Peterlin.** 1987. Human immunodeficiency virus long terminal repeat responds to T-cell activation signals. Proc. Natl. Acad. Sci. USA **84**:6845–6849.
59. **Ullman, K. S., J. P. Northrop, A. Admon, and G. R. Crabtree.** 1993. Jun family members are controlled by a calcium-regulated, cyclosporin-A-sensitive signaling pathway in activated T lymphocytes. Genes Dev. **7**:188–196.
60. **Valerie, K., A. Delers, C. Bruck, C. Thiriart, H. Rosenberg, C. Debouck, and M. Rosenberg.** 1988. Activation of human immunodeficiency virus type 1 by DNA damage in human cells. Nature (London) **333**:78–81.
61. **Viscidi, R. P., K. Mayur, H. M. Lederman, and A. D. Frankel.** 1989. Inhibition of antigen-induced lymphocyte proliferation by Tat protein from HIV-1. Science **246**:1606–1608.
62. **Vogel, J., S. H. Hinrichs, R. K. Reynolds, P. A. Luciw, and G. Jay.** 1988. The HIV tat gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. Nature (London) **335**:606–611.
63. **Wano, Y., M. Feinberg, J. B. Hosking, H. Bogerd, and W. C. Green.** 1988. Stable expression of the tax gene of type I human T-cell leukemia virus in human T cells activates specific cellular genes involved in growth. Proc. Natl. Acad. Sci. USA **85**:9733–9737.