Human immunodeficiency virus type 1 (HIV-1) infection produces a state of immunological dysregulation which includes a state of hyperactivation of B and T cells with a general increase in cytokine production (52). These alterations are possibly necessary for the maintenance of virus infection and may be at least partially explained by a direct influence of virus-encoded products on the mechanisms that control immune cell activation.

The transactivator protein Tat of HIV-1 is required for efficient transcription and viral replication (18, 30). Tat binds to the transactivation response (TAR) element, an RNA stem-loop located from positions +1 to +59 of the HIV-1 long terminal repeat (LTR) and exerts its function mainly by increasing the efficiency of transcription elongation (16, 35, 42). Although the exact mechanism by which Tat enhances the rate of transcription elongation is still unknown, it is now clearly established that Tat interacts with different host proteins which are necessary for Tat function. Among those factors, proteins belonging to the general transcription machinery of the host cell have been reported to interact with Tat. These proteins include the core RNA polymerase II (13, 44), whose C-terminal domain is required for Tat-mediated transactivation (50), TAFII55 (12), and TFIIH and CDK7 (5, 14). Tat has also been reported to interact with other nuclear kinase complexes (21, 68), with the transcription factor Sp1, which binds to three tandem sites in the core enhancer element of the HIV-1 LTR (29), and with cyclin T, which has recently been identified as a TAR RNA-binding cofactor for Tat (66). In addition to its role in HIV-1 transcription, Tat has also been shown to up-regulate the transcription of several host genes such as those encoding tumor necrosis factor alpha (7), interleukin 2 (IL-2) (51, 63), and IL-6 (2) by interacting with different cellular factors and contributing to some of the altered cytokine production which occurs after HIV-1 infection. Some reports have mapped those Tat-mediated effects to sites that can potentially bind the nuclear factor of activated T cells (NFAT) in genes whose expression is regulated by NFAT proteins (51, 63).

NFAT1 (also called NFATp) (46) is the founding member of the NFAT family of transcription factors, which plays a key role in inducible gene transcription during the immune response (56, 57). Other members of the NFAT family have been described and termed NFAT2 (also called NFATc) (49), NFAT3 (23), and NFAT4 (also called NFIX) (23, 43), with each one having a specific tissue distribution and function. NFAT proteins are activated by stimulation of receptors which induce calcium mobilization and also by calcium ionophores such as ionomycin. Calcium mobilization activates calcineurin, which causes dephosphorylation and subsequent nuclear translocation of NFAT proteins (38), which cooperate in the nucleus with members of the Fos and Jun families of transcription factors (28). This process can be inhibited by the immunosuppressants cyclosporine A (CsA) and FK506, which inhibit calcineurin activity (61). NFAT proteins are involved in the regulation of numerous activation-associated genes that encode cytokines, transcription factors, cell surface receptors, and other signaling proteins (56, 57). The amino-terminal domain of NFAT1 contains its major transactivation domain (40), which is followed by the calcineurin-binding regulatory domain and the DNA-binding domain (DBD), which has similarity at both the sequence and structural levels with Rel proteins (11, 27). Both domains are highly conserved among the different members of the NFAT family (27, 39). NFAT1 is expressed in several immune system cells, including T cells and monocytes (57), as well as in other nonimmune tissues such as the central nervous system (22), all of which are potential targets for HIV-1 infection.

The enhancer element of the HIV-1 LTR contains two tandem NF-kB sites whose function seems to be essential for HIV-1 transcription in both T cells and monocytes (1, 26). NFAT proteins have been shown to bind these sites, and an activating
role for one of the NFAT family members, NFATc/NFAT2, in HIV-1 LTR transcription mediated through the κB sites and in HIV-1 replication has recently been described (32, 33). However, other studies have found that in HIV-1 viruses with mutations in the gag gene which render viral replication independent of cyclophillin A, treatment of infected cells with CsA had no inhibitory effect on HIV-1 replication and at some concentrations even produced stimulation of virus replication (6).

In this paper, we have examined the role of the NFAT family member NFAT1 in HIV-1 pathogenesis. We demonstrate that both Tat and NFAT1 bind to these two promoter elements and that Tat exerts a negative effect on LTR transcription mediated by NF-κB.

MATERIALS AND METHODS

Plasmids. The expression plasmids pEFG-TatNFAT1-C, which bears the gene encoding a hemagglutinin (HA)-tagged murine NFAT1, and pGAL4-NFAT1 (1–415), which bears the gene encoding a fusion protein containing the DBD of Gal4 and amino acids 1 to 415 of NFAT1 and pGAL4-ΔSp2, have been previously described (39, 40). The expression plasmids pEFG-TatNFAT1(1–415) and pEFG-TatNFAT1DBD bear the DNA sequences encoding amino acids 1 to 415 and 1 to 415 of the DBD (amino acids 398 to 694) of NFAT1, respectively (27, 40). pC-Tat expresses HIV-1 Tat protein under the control of the cytomegalovirus promoter, and pEFG-Tat C226G expresses a mutant Tat with a substitution of Gly for Cys226. pDNA3-mRelA, which expresses the murine RelA protein under the control of the cytomegalovirus promoter, was a gift from Sankar Ghosh (Yale University). The GAL4 luciferase reporter plasmid GAL4-Luc (10) was kindly provided by Stephen C. Harrison. The luciferase reporter vector NFAT3-B-Luc, containing three copies of the GAL4 repeat, was made by PCR-mediated mutagenesis of HIV-1 LTR-Luc with NF-κB oligonucleotides (Strategene). In this plasmid the sequences of both κB sites of the HIV-1 LTR were changed to GG GCCATTTCC to GGGGACTAGTT. The luciferase reporter vector NFAT3-B*-Luc, containing three copies of the Gal4 repeat, was made by PCR-mediated mutagenesis of HIV-1 LTR-Luc with P6 oligonucleotides (Strategene). In this plasmid the sequences of both κB sites of the HIV-1 LTR were released from GG GCCATTTCC to GGGGACTAGTT. The luciferase reporter vector NFAT3-B-Sp1 wild type, 5′-GGCGCAGGTTCCAGGATGCACCGTTCCGGCGG; 5′-GGCGCAGGTTCCAGGATGCACCGTTCCGGCGG; and NFAT3-B-Sp1 mutant, 5′-GGCGCAGGTTCCAGGATGCACCGTTCCGGCGG; and 5′-GGCGCAGGTTCCAGGATGCACCGTTCCGGCGG. A specific effect on LTR transcription mediated by NF-κB has been observed with six-His-tagged fusion proteins and purified Tat proteins on a SDS-polyacrylamide gel and analyzed by Western blotting with anti-67.1 and anti-72 (22, 46), which recognize different epitopes in the amino-terminal domain of NFAT1, or with R59 (46), which is directed against the NFAT1 DBD. In every assay a binding reaction mixture with GST protein was included as a negative control.

Immunoprecipitations. Cellular extracts from HEK293T cells transfected with pC-Tat and/or pEFG-TatNFAT1-C were obtained by lysing the cells in a buffer containing 150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 0.25% Nonidet P-40, 0.5 mM EDTA, and 5 mM DTT in a total volume of 500 μl. GST-Tat proteins (to 8 μg) bound to glutathione-Sepharose beads (Pharmacia) were incubated for 4 h at 4°C with 250 to 500 ng of purified six-His NFAT1 proteins. After the incubation, the beads were washed three times with the same buffer and bound proteins were separated on a sodium dodecyl sulfate (10%)–12% polyacrylamide gel and analyzed by Western blotting with anti-67.1 and anti-72 (22, 46), which recognize different epitopes in the amino-terminal domain of NFAT1, or with R59 (46), which is directed against the NFAT1 DBD. In every assay a binding reaction mixture with GST protein was included as a negative control.

RESULTS

HIV-1 Tat enhances NFAT1-driven transcription. As HIV-1 Tat had been shown to play a role in the regulation of numerous cytokine genes cooperating with different cellular factors (2, 7, 51, 63), we asked whether it could affect transactivation by NFAT1, a transcription factor involved in regulating the expression of a large number of cytokine genes (57). For that purpose, we studied the effect of HIV-1 Tat on NFAT1-driven transcription in transient-transfection experiments with Jurkat cells. When expressed alone, HIV-1 Tat had little effect on the basal activity of NFAT3-X-Luc, which contains three copies of a canonical NFAT1-API site. However, cotransfection of Tat potentiated the activity of NFAT1 to drive reporter expression (Fig. 1A). To exclude a possible effect of Tat on API proteins and to check if this result could be reproduced with only the transactivation domain of NFAT1, a series of experiments were carried out with a fusion protein containing the GAL4 DBD and the first 415 amino acids of NFAT1, which contains its major transactivation domain (40). Tat expression had no effect on the activity of the GAL4 reporter plasmid but significantly (P < 0.02) upregulated GAL4-dependent transactivation mediated by the first 415 amino acids of NFAT1, which contains its major transactivation domain (40). Tat expression had no effect on the activity of the GAL4 reporter plasmid but significantly (P < 0.02) upregulated GAL4-dependent transactivation mediated by the first 415 amino acids of NFAT1, which contains its major transactivation domain (40). Transactivation of Tat was determined using a direct protein-protein interaction between the NFAT1 amino-terminal region and Tat, we performed in vitro binding experiments with GST-Tat (one or two exons) and different six-His-tagged fragments of NFAT1. Both forms of Tat protein were able to pull down a fragment of NFAT1 containing its first 415 amino acids (Fig. 1C), although the ability of the Tat two-exon protein to bind NFAT1 appeared somewhat greater than that of the one-exon

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Expression and purification of recombinant proteins. The NFAT1 DBD [pNFATpKX5(1–297) (27)] and different fragments from the amino-terminal do-
protein (compare lane 3 to lane 4 in Fig. 1C). When the DBD of NFAT1 was used in similar experiments, no binding to Tat was detected, indicating that the interaction of these two proteins involved specifically the amino-terminal domain of NFAT1 (Fig. 1D). Furthermore, in EMSAs carried out with nuclear extracts from Cl.7W2 cells and an IL-2 promoter ARRE2 site probe, a new slower-migrating complex could be observed when purified GST-Tat was added to the binding
reaction mixture (Fig. 1E, compare lanes 1 and 2); this complex was supershifted with antibodies to NFAT1 and HIV-1 Tat (Fig. 1E, lanes 4 and 5). As expected from the lack of interaction of the NFAT1 DBD with Tat (Fig. 1D), no new complex was detected in EMSAs when the binding reaction mixtures contained a combination of the purified NFAT1 DBD and GST-Tat (data not shown).

To confirm that NFAT1 interacted with Tat in cells, HEK 293T cells were cotransfected with plasmids expressing HA-tagged NFAT1 and/or HIV-1 Tat. Cells were stimulated with PMA and ionomycin for 6 h to localize NFAT1 to the nucleus, and total cellular extracts were immunoprecipitated with antibodies to Tat or the HA tag. Antibodies against HIV-1 Tat co-precipitated NFAT1 only in cells which had been cotransfected with both plasmids (Fig. 1F), similarly, anti-HA antibodies co-precipitated HIV-1 Tat together with HA-NFAT1 (Fig. 1G).

No Tat was immunoprecipitated with the anti-HA antibody in the absence of HA-tagged NFAT1 (Fig. 1G, lane 3), thus ruling out nonspecific binding of HIV-1 Tat to the anti-HA antibody. These experiments demonstrated the existence of an NFAT1–HIV-1 Tat interaction in vivo.

NFAT1 HIV-1 Tat interaction is mediated by the transactivation domain of NFAT1 and the amino-terminal region of Tat.

To localize more precisely the region of the NFAT1 amino-terminal domain involved in the interaction with Tat, we expressed different fragments of this region and tested their capacity to bind Tat. In vitro binding experiments showed that only the most amino-terminal fragment (amino acids 1 to 96) was able to bind Tat (Fig. 2A, lane 12) and that fragments containing C-terminal regions of the NFAT1 amino-terminal domain (amino acids 67 to 415 and 140 to 415) showed no Tat binding activity (Fig. 2A, lanes 2 and 9). Therefore, the region of NFAT1 which interacted with Tat was localized to the first 96 amino acids of NFAT1, which constitute the major, strongly acidic, transactivation domain of NFAT1 (40).

If the transactivation domain is the region of NFAT1 that makes contact with Tat, the transactivational activity of a fusion protein containing only this domain should also be enhanced by Tat. To test this hypothesis, transient-transfection experiments were performed with Jurkat cells and a GAL4 fusion protein containing an NFAT1 amino-terminal domain with a deletion from amino acids 145 to 387 (GAL4-ΔSP2) and therefore lacking the regulatory domain but maintaining the major transactivation domain. As predicted, coexpression of Tat upregulated GAL4-ΔSP2-mediated transcription from a GAL4 luciferase reporter (Fig. 2B).

We also determined the region of the HIV-1 Tat domain involved in making contact with the transactivation domain of NFAT1. In vitro binding experiments revealed that the NFAT1-Tat interactions involved residues contained in the first 26 amino acids of Tat, since a GST-Tat fusion protein with a deletion of amino acids 2 to 26 did not bind NFAT1 (Fig. 3A, compare lanes 2 and 5). However, a single point mutation in this region, C22 to G, that produces a transcriptionally inactive Tat, was still able to bind the amino-terminal domain of NFAT1 (Fig. 3A, lane 4), suggesting that the interactions of Tat with NFAT1 involved a domain of Tat different than that involved in the interactions of Tat with cyclin T, P-TEFb, or other components of the basal transcriptional machinery (13, 66, 68).

We sought to determine whether the functional cooperativity of Tat with NFAT1 required a transcriptionally competent Tat protein by using an inactive Tat protein in which Cys22 had been mutated to Gly. As predicted, no enhancement of NFAT1-driven transcription was observed when this mutant Tat protein was coexpressed with a GAL4-NFAT1(1–415) fusion protein (Fig. 3B). The level of expression of the mutant Tat protein, checked by Western blotting with anti-Tat antibodies, was similar to the levels obtained with the vector expressing wild-type Tat (data not shown). In a second approach, we tested the effect of blocking NFAT1-Tat interaction by overexpressing the first 27 amino acids of Tat as a GFP fusion protein. We predicted that this fragment, which contained the region of Tat involved in the interaction with NFAT1, would displace Tat from its binding site on NFAT1 and therefore would abolish NFAT1-Tat cooperativity. Confirming our hypothesis, cotransfection of a plasmid expressing GFP-Tat(1–27) inhibited Tat enhancement of NFAT1-mediated transactivation (Fig. 3C).
NFAT1 inhibits Tat-mediated activation of HIV-1 LTR transcription. Having shown that the NFAT1-Tat interaction potentiated the transcriptional activity of NFAT1, we asked whether, conversely, NFAT1 would modulate Tat-mediated activation of the HIV-1 LTR (Fig. 4). Transfection assays were performed with Jurkat cells, and as previously reported, Tat expression resulted in a large increase in CAT activity driven by the HIV-1 LTR in transiently transfected Jurkat cells. This increase was significantly reduced ($P < 0.01$) by addition of NFAT1 (Fig. 4A). This effect showed a direct correlation with the amount of NFAT1 plasmid cotransfected (Fig. 4B). In these experiments, cells were stimulated with PMA and ionomycin to achieve activation and translocation of NFAT1 to the nucleus (38, 39, 61), but the concentration of PMA used in these experiments (10 nM) produced no detectable activation of the HIV-1 LTR, ruling out the possibility that NFAT1 was depressing Tat-mediated activation by downregulating NF-κB or other factors involved in Tat-independent activation of the HIV-1 LTR.

To determine whether NFAT1 binding to DNA was necessary for downregulation of Tat activity, we took advantage of the fact that NFAT1 bound preferentially to the 3′ half of the NF-κB sites of the HIV-1 LTR (Fig. 5A). Both full-length NFAT1 (lane 1) and a recombinant fragment comprising the DBD of NFAT1 (lane 5) showed binding to a radiolabeled NF-κB oligonucleotide (−66 to −91). As previously shown for the immunoglobulin κ enhancer κB site (45), the recombinant NFAT1-DBD formed two complexes with the probe, which contained one (lower complex) or two (upper complex) molecules of NFAT1-DBD (Fig. 5A, lane 5). Binding of both full-length NFAT1 and NFAT1-DBD was specific, as judged by competition with excess unlabeled wild-type and mutated oligonucleotides (data not shown). NFAT1 bound to the same level (Fig. 5A, lanes 2 and 6) to an oligonucleotide with a mutation in the 5′ half of the κB site, which is known to abolish NF-κB binding (54), but showed greatly reduced binding to an oligonucleotide with a mutation in the 3′ half of the κB site (Fig. 5A, lanes 3 and 7). We then constructed an HIV-1 LTR luciferase reporter plasmid in which both κB sites had been mutated in their 3′ halves to abolish NFAT1 binding (Fig. 4). When this reporter plasmid was used, NFAT1 expression caused no significant reduction of luciferase activity in Tat-transfected cells (Fig. 5B), suggesting that NFAT1-mediated downregulation of Tat activity requires positioning of NFAT1 on the κB sites of the HIV-1 LTR.

NFAT1 competes with NF-κB for the binding to the κB sites of the HIV-1 LTR. Since NFAT1 bound the κB sites of the HIV-1 LTR but inhibited Tat-mediated transactivation, we also tested the effect of NFAT1 on NF-κB-mediated activation of the HIV-1 LTR. A different NFAT family member, NFAT2,
has been shown to synergize with NF-κB for HIV-1 replication and transactivation of the HIV-1 LTR (32, 33). In contrast, we found that NFAT1 inhibited RelA-mediated transactivation of the HIV-1 LTR (Fig. 6A). Transfection of a RelA expression plasmid into Jurkat cells produced an increase in the CAT activity of the HIV-1 LTR-CAT reporter vector, which was significantly reduced in a dose-dependent manner by expression of NFAT1 (Fig. 6A). A possible explanation for this effect is that NFAT1 and NF-κB compete for binding to the κB sites of the HIV-1 LTR (Fig. 6B). A labeled κB-Sp1 probe was incubated with 2 ng of NF-κB p50 in the presence of increasing amounts of p50-κB and increasing amounts of NFAT1 DBD. The complex formed by p50-κB disappeared as higher concentrations of NFAT1 DBD were added to the reaction mixture, with the appearance of new bands corresponding to the NFAT1 DBD monomer and dimer (Fig. 6B).

FIG. 5. NFAT1 binding to the κB site of the HIV-1 LTR is necessary for NFAT1-mediated downregulation of Tat transactivation. (A) Nuclear extracts from ionomycin-stimulated C17.2W2 cells (lanes 1 to 4) or the purified NFAT1 DBD (lanes 5 to 8) were incubated with radiolabeled oligonucleotides containing the adjacent κB and Sp1 sites of the HIV-1 LTR (wild type, lanes 1 and 5) or the indicated 5' or 3' mutations (Mut). Arrows indicate the positions of the different complexes. Sequences of the oligonucleotides used in the binding assays are shown below. Sequences in boldface type indicate the actual κB and Sp1 sites on the probe. Mutated bases in the oligonucleotides with 5' or 3' mutations are underlined. (B) Jurkat cells were transfected with Tat (0.25 μg) and/or NFAT1 (10 μg) expression plasmids and with luciferase reporter vectors containing a mutated LTR in which both κB binding sites were made unable to bind NFAT1 by mutating their 3' halves from TTCC to AGTT. The effect of NFAT1 on the transactivation caused by Tat was assayed. Results are shown as percentages of the luciferase activity of the pcTat-transfected cells. Values are the means of results from two independent experiments. In all the experiments, the total amounts of DNA were maintained at a constant level by cotransfecting balancing amounts of empty pEFTag plasmid.

FIG. 6. NFAT1 competes with NF-κB for binding to the κB site and downregulates NF-κB-mediated activation of the HIV-1 LTR. (A) RelA-mediated activation. In the left graph, Jurkat cells were cotransfected with 1 μg of the reporter plasmid HIV-1 LTR CAT and expression plasmids for NFAT1 (10 μg) and/or RelA (3 μg). Cells were stimulated for 12 h with 10 nM PMA and 2 μM ionomycin. Results are shown as percentages of the CAT activity of the RelA-transfected cells. Values are means ± standard errors of results from six independent experiments. **, P < 0.01. In the right graph, Jurkat cells were cotransfected with 1 μg of the reporter plasmid HIV-1 LTR CAT, 3 μg of a RelA expression plasmid, and increasing amounts of an NFAT1 expression plasmid. Twenty-four hours after the transfection, cells were stimulated for 12 h with 10 nM PMA and 2 μM ionomycin. A representative experiment is shown. In all the experiments the total amounts of DNA were maintained at a constant level by cotransfecting balancing amounts of empty pEFTag plasmid. (B) A labeled oligonucleotide containing the adjacent κB and Sp1 sites of the HIV-1 LTR was incubated with 2 ng of NF-κB p50 in the presence of increasing amounts of the purified recombinant NFAT1 DBD. Arrows indicate the positions of the different complexes.
detected at any of the tested concentrations of NFAT1. These binding experiments showed that NF-κB1 and NFAT1 could not bind simultaneously to the same κB sites but that they seemed to compete for them.

**DISCUSSION**

The viral protein Tat is essential for HIV-1 gene expression and replication and its function is regulated by interactions with several host factors. In addition to being a potent activator of HIV-1 gene transcription, Tat may regulate the expression of other cellular genes whose products influence the course of viral infection, although the exact mechanism underlying most of these effects is still unknown. Specifically, HIV-1 infection is known to produce an altered pattern of cytokine production from both T cells and monocytes (47, 52, 67), and several reports have described a direct involvement of Tat in the regulation of cytokine genes. Expression of IL-6 is induced by an interaction of Tat with the CAAT enhancer binding protein beta (2), and a direct participation of Tat in IL-2 expression has also been reported (51).

In this paper we have addressed the possibility that one of the mechanisms responsible for Tat-mediated regulation of host cellular genes involves the transcription factor NFAT1. We have shown that Tat upregulates NFAT1 transcriptional activity and that the amino-terminal domain of NFAT1 is sufficient for this effect. This result is consistent with previous observations indicating that the increase in IL-2 expression caused by Tat maps to the NFAT sites of the IL-2 promoter (63) and to the CD28 response element (51), which is known to bind either NFAT or Rel proteins (41, 59, 60). Our results show that NFAT1 is able to bind Tat in vivo and that this interaction takes place between the major transactivation domain of NFAT1 and the amino-terminal region of Tat. Thus, the ability of NFAT1 to recruit Tat to the regulatory regions of cytokine genes may promote the interaction of Tat with TFIID, RNA polymerase II, or other transcription factors or kinases in the cooperative enhancer complex, thus promoting the transcription of these genes. As NFAT1 is a key regulator of gene expression during the immune response (57), the Tat-NFAT1 interaction may be responsible for the upregulation of genes encoding cytokines and other immune modulators during HIV-1 infection (51).

Transient transfection of Jurkat T cells with NFAT1 and Tat-expressing plasmids indicates that NFAT1 inhibits Tat-mediated transactivation of the HIV-1 LTR. Although this effect can be observed by overexpressing the isolated terminal domain of NFAT1 (data not shown), it is augmented by binding of NFAT1 to the κB sites of the HIV-1 LTR. The degree of downregulation produced by NFAT1 coexpression is greater than 50%. Studies performed with different Tat mutants have revealed that a 50% reduction in Tat transcriptional activity suffices for significant impairment of HIV-1 gene transcription and virus replication (65). Many other proteins have been identified as Tat-binding proteins, and some of these, such as Oct2 and p53, have been described as negative regulators which produce degrees of inhibition of transcription of the HIV-1 LTR similar to that produced by NFAT1 (36, 37). The inhibitory effect of NFAT1 on HIV-1 LTR transcription is also consistent with the fact that in mutant HIV-1 viruses which do not require virion-associated cyclophilin A to initiate infection, CsA, which inhibits NFAT1 translocation into the nucleus, can have a stimulatory effect on virus replication (6). The mechanism of NFAT1-mediated Tat inhibition remains to be investigated: it may involve a direct squelching or blocking of Tat-mediated transactivation by NFAT1, or alternatively, the NFAT1-Tat interaction may block the interaction of Tat with other host factors required for Tat to exert its function through the TAR element (18, 66).

Recent reports have indicated that another member of the NFAT family, NFAT2, activates both HIV-1 gene expression and replication (32, 33). In contrast, our results indicate that NFAT1 has a largely downregulatory effect on HIV-1 LTR expression in Jurkat T cells. Differences in the mechanisms of regulation and functions of these two family members may account for their distinct effects on HIV-1 regulation. Indeed, NFAT1 and NFAT2 have been postulated to have opposite effects on T-cell function, based on the immune phenotype of cells lacking either of these two transcription factors. While T cells lacking NFAT1 show a maintained high level of IL-4 after stimulation, which indicates a role for NFAT1 in downregulating IL-4 transcription and thus inhibiting T-helper 2 responses (31), T cells lacking NFAT2 show an impairment in IL-4 production, suggesting a positive role for this family member in IL-4 gene transcription (55). T cells and other immune cells may selectively use different members of the NFAT family to regulate gene expression of HIV-1 and other genes involved in the immune response. Alternatively, the observed differences may result from a hierarchy of transcriptional activity (NF-κB > NFAT2 > NFAT1), and NFAT1 might be capable of upregulating HIV-1 and IL-4 gene expression, especially in cell types (e.g., naive primary T cells) lacking high-level expression of NF-κB and NFAT2.

The fact that NFAT1 and Tat have opposite effects on each other’s activities is not surprising, as a similar regulatory interplay between Tat and Sp1 has been described. A cooperative interaction between NF-κB bound to the κB sites and Sp1 bound to the adjacent Sp1 sites is required for HIV-1 gene expression (53), and Sp1 is also essential for Tat-mediated activation of the HIV-1 LTR (4, 62). However, Tat inhibits the transcription of several Sp1-activated cellular promoters by acting directly or indirectly on Sp1 or Sp1-like proteins bound to their specific binding sites in those promoters (25).

The κB sites of the HIV-1 LTR are essential for viral gene expression and replication in T cells and monocytes (1, 26). We have demonstrated that NFAT1 and NF-κB do not bind cooperatively to the κB sites of the HIV-1 LTR but rather that they compete for binding. Consistent with this observation, NFAT1 inhibits RelA-mediated transactivation of the HIV-1 LTR, presumably by competing with NF-κB for occupancy of the κB sites. A similar interplay between NFAT and NF-κB proteins occurs on the human IL-4 promoter, where the NFAT1-mediated activation of the IL-4 promoter in CD4+ cells is negatively controlled by competitive binding of RelA to the P element on this promoter (9). Thus, the NFAT–NF-κB competition for the κB sites of the HIV-1 LTR may be another example of a more general regulatory mechanism used by cells to modulate the activities of these two families of transcription factors. Other proteins such as HMG-I(Y), which are known to differentially regulate the binding affinities of NFAT and Rel proteins to specific DNA sites, may also be involved in such a mechanism (34). It has been shown that Stat2 modulates HIV gene expression by competing for the coactivator p300 with NF-κB (24); similarly, NFAT1 may also compete with NF-κB for transcriptional coactivators such as p300 (17, 24), whose cellular concentration may be limited.

In summary, we have shown the existence of an interaction between the major transactivation domain of NFAT1 and the amino-terminal region of HIV-1 Tat. NFAT1 interaction with Tat results in potentiating of NFAT1-mediated transactivation; conversely, NFAT1 inhibits both Tat-mediated and RelA-mediated HIV-1 transcription. Other reports have shown that
NFAT2 potentiates HIV-1 replication and gene transcription expression (32, 33). Notably, the immune phenotype of T cells deficient in NFAT1 and NFAT2 suggests that these related transcription factors have opposing effects on IL-4 gene transcription as well (55). The contrasting effects of NFAT1 and NFAT2 suggest that NFAT transcription factors exert complex modulatory effects on HIV-1 transcription and the immune response.

ACKNOWLEDGMENTS

We thank S. Ghosh, S. C. Harrison, D. J. McKean, M. Montminy, and H. Okamura for their generous gifts of reagents. The following reagents were obtained through the AIDS Research and Reagent Reference Program, Division of AIDS, NIH, NIAID, H: GST–Tat-1 86R and GST–Tat-1 72R from A. Rice; antisera against HIV-1 Tat from B. Cullen; and recombinant immunodominant antibody against HIV-1 Tat from the Division of AIDS, NIAID.

This work was supported by NIH grant CA42471 and a Leukemia Society of America scholar award (to A.R.). F.M. was supported by a postdoctoral fellowship from the Ministry of Education and Culture of Spain.

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