Human T cell transcription factor GATA-3 stimulates HIV-1 expression

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ABSTRACT

A family of transcriptional activating proteins, the GATA factors, has been shown to bind to a consensus motif through a highly conserved C₄ zinc finger DNA binding domain. One member of this multigene family, GATA-3, is most abundantly expressed in T lymphocytes, a cellular target for human immunodeficiency virus type 1 (HIV-1) infection and replication. In vitro DNase I footprinting analysis revealed six hGATA-3 binding sites in the U3 region (the transcriptional regulatory domain) of the HIV-1 LTR. Cotransfection of an hGATA-3 expression plasmid with a reporter plasmid whose transcription is directed by the HIV-1 LTR resulted in 6- to 10-fold stimulation of LTR-mediated transcription, whereas site specific mutation of these GATA sites resulted in virtual abrogation of the activation by hGATA-3. Further, deletion of the hGATA-3 transcriptional activation domain abolished GATA-dependent HIV-1 *trans*-activation, showing that the stimulation of viral transcription observed is a direct effect of cotransfected hGATA-3. Introduction of the HIV-1 plasmids in which the GATA sites have been mutated into human T lymphocytes also caused a significant reduction in LTR-mediated transcription at both the basal level and in (PHA- plus PMA-) stimulated T cells. These observations suggest that in addition to its normal role in T lymphocyte gene regulation, hGATA-3 may also play a significant role in HIV-1 transcriptional activation.

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of acquired immune deficiency syndrome (AIDS), and infects human CD4⁺ T cells and myelomonocytic cells using the CD4 receptor for viral entry (1, 2, 3). The retroviral long terminal repeat (LTR) of HIV-1 has been suggested to play a number of roles in the regulation of the life cycle of the virus after infection (4). The viral LTR U3 region contains a variety of cis-acting regulatory sequences responsible for modulating viral gene expression and was initially divided into the basal promoter

region, the enhancer (Enh), the negative regulatory element (NRE) and the downstream regulatory element (4).

Beginning at the transcription initiation site of the HIV-1 LTR, the leader binding protein (LBP-1) has been shown to interact with three sites between –17 and +27, and negatively regulates the expression of HIV-1 (5); the virally-encoded regulatory factor *tat* also binds to this same region in both DNA and RNA (6, 7, 8). The minimal promoter contains TATA (9) and Sp1 (10, 11) factor binding sites responsible for basal level transcription of the virus. The enhancer element contains two binding sites for NF- κ B (12), a ubiquitous transcription factor which becomes post-translationally induced upon T cell activation, and is the principal *cis*-determinant for the inducibility of HIV-1 transcription by T cell stimuli. Lying further 5' from the HIV-1 CAP site is the NRE region, which suppresses transcription of HIV-1 by two- to three-fold (4). The NRE region contains recognition sequences for a variety of cellular transcription factors, including three AP-1 factor binding sites (13), a consensus *myb* binding site (14), a T cell-specific NFAT-1 binding site (15), and another element (at –165 to –175) which closely resembles the negatively regulatory element of the IL-2 gene (16).

While the transcriptional regulation of HIV-1 has been extensively examined, the precise role that many of these factors may play in the basal or induced levels of transcriptional stimulation from HIV-1 remains obscure, primarily due to a lack of direct evidence for the proposed activity of these factors in several of the aforementioned cases. Moreover, the only cell type-specific transcription factor known to bind to this LTR (NFAT-1) has been shown not to participate in activation of the virus (45). We nonetheless anticipated that hematopoietic cell-restricted transcription factors might play an important role in HIV-1 gene regulation.

The T lymphocyte lineage-restricted transcription factor, human GATA-3 (hGATA-3), is a member of a multigene family which binds to a consensus GATA DNA sequence motif through a highly conserved C₄ zinc finger DNA binding domain (17, 18). GATA-3 is predominantly expressed in T lymphocytes, where no other GATA proteins are detectable (19), as well as in a specific subset of neurons within the vertebrate CNS and

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in erythroid cells (18, 19, 20), and is highly conserved in amino acid sequence identity when comparing the vertebrate GATA-3 proteins (18, 21). GATA-3 binding sites have been identified in the regulatory regions of the T cell receptors (TCRs; 22, 23, 24, 25, 26), and it has been shown that the GATA-3 sites within the human TCR δ gene enhancer are required for the tissue-specific activation of this gene (18, 27).

On inspection of the transcriptional regulatory domain of the HIV-1 LTR, we found four consensus GATA binding sites (WGATAR; 28), and therefore sought to determine whether or not hGATA-3 might be involved in regulating transcription of the virus. hGATA-3 was found to bind to the four anticipated GATA sites, but in addition, strongly bound *in vitro* to two other non-consensus sites. Mutation of all sites resulted in much lower activation of the virus upon transfection into T lymphocytes. These effects were shown to be specific for the hGATA-3 protein by introduction of mutations into the coding sequence of the hGATA-3 transcriptional activation domain; while the mutant factor binds to DNA indistinguishably from the wild type hGATA-3 protein, it no longer stimulates HIV-1 LTR-directed transcription. These data show that transcription factor hGATA-3 contributes to transcriptional activation by the HIV-1 LTR.

MATERIALS AND METHODS

DNA binding assays

10 μ g of RSV/hGATA-3 cDNA was transfected into QT6 quail fibroblast cells using CaPO_4 (detailed below) and cell lysates were prepared 48 hours after transfection (29); QT6 cells were used for expression of GATA proteins, since they lack discernible GATA binding activities (17 and Figure 2C). The cell lysates were then partially purified for total DNA binding activity by passing through, and high salt elution from, a double stranded calf thymus DNA column and pooling the bound fractions (30). DNase I footprint assays were performed by incubating this partially purified hGATA-3 protein with either coding or non-coding strand end-labeled HIV-1 LTR DNA (30). For electrophoretic gel mobility shift assay (EGMSA), whole-cell extracts prepared as above were incubated with radiolabeled oligonucleotide T δ E4 (18), which corresponds to the GATA sequences within human T cell receptor δ gene enhancer footprint 4 (24). Gel shift assays were performed as described previously (18).

PCR mutagenesis

The primers used in these PCR assays (each of which contain additional nucleotides outside of the restriction site for convenience of subsequent restriction enzyme digestion) are shown in Table 1. The mutation of each GATA binding site (determined in the footprinting assays, above) in the HIV-1 LTR was independently generated by PCR mutagenesis into the corresponding restriction enzyme site shown in Fig. 2B. DNA sequences 5' and 3' to the target GATA site were amplified separately, digested with the appropriate restriction enzyme, and ligated into pCAT-basic vector (Promega). Further mutations were introduced by repeating the procedure until all of the individual GATA site mutations were incorporated into the noGATA/CAT plasmid. Since sequences 5' to GATA binding site 5 (Figure 1A) were found to not influence the transcriptional activity of the HIV-1 LTR (4, 31), both HIVwt/CAT and the noGATA/CAT test plasmids extend to nucleotide -376 relative to the mRNA CAP site.

A mutation which specifically abolishes all *trans*-activation activity within the hGATA-3 protein coding sequence was generated by removing an internal SmaI fragment (31). The mutant hGATA-3 plasmid (called d29-128) specifically deletes, in frame, amino acids 29 to 128 of the hGATA-3 protein (18), and was confirmed by DNA sequencing. The mutant d29-128 protein binds to DNA in a manner indistinguishable from wild-type hGATA-3.

Transfections

HeLa cells were transfected using the calcium phosphate procedure (32) with 10 μ g of the wild type or mutated HIV-1 LTR constructs plus 5 μ g of RSV/hGATA-3 (18). 40% of the cells from a confluent plate were used for each transfection. Cell lysates were prepared 48 hours after transfection by the freeze/thaw procedure (33). Protein concentrations were determined and CAT assays were performed using aliquots of extract containing equal quantities of recovered protein (34). The result was quantified by determining the amount of acetylated [^{14}C] chloramphenicol produced in the enzymatic assay and the degree of conversion was quantitated on a Molecular Dynamics PhosphorImager.

Jurkat cells were grown in RPMI 1640 containing 10% fetal bovine serum. 10^7 cells were transfected with 5 μ g of HIV-1 LTR constructs using DEAE-dextran (35). 100 ng of RSV/luciferase was also added as a transfection efficiency control. Half of the cells from each transfection were grown in the presence of 10 nM phorbol myristate acetate (PMA; Sigma) plus 2 μ g/ml phytohemagglutinin (PHA; Sigma) for the last 20 hours in order to activate the T lymphocytes. Cell lysates were prepared 44 hours after transfection and luciferase expression was quantitated with a luminometer. CAT assays were normalized using an amount of extract containing equal luciferase activity.

All cell transfection or cotransfection experiments reported here were performed on at least three separate occasions, often using different plasmid preparations. Error bars represent one s.d. from these accumulated assays.

RESULTS

hGATA-3 binds *in vitro* to six sites within the HIV-1 LTR

Since the expression profile of hGATA-3 is partially consistent with the documented tropism of HIV-1 replication, one might anticipate that hGATA-3 could play a role in the T lymphocyte expression of HIV-1. Initial inspection of the sequences in the viral LTR indicated the presence of four potential GATA factor binding sites (Figure 1A). Both strands of HIV-1 U3 region DNA were examined for their ability to bind to hGATA-3 by DNase I footprinting analysis using partially purified hGATA-3 protein expressed in, and partially purified from, animal cells (Materials and Methods; as also shown in Figure 2C, no GATA binding activity is normally detected in QT6 cells). The results actually revealed six hGATA-3 interaction sites (Figure 2A), all localized within the 5' domain of the LTR. The four consensus GATA sites (28) were protected by hGATA-3, as were two non-canonical binding sites: GATTA (site 5) and GATGA (site 2; Figure 1A). The latter site overlaps a previously defined binding site for a 50 kD negative regulatory protein of the IL-2 gene (16).

The specificity for binding of hGATA-3 to these sites was confirmed in competition experiments. When a 50-fold molar excess of the high affinity GATA oligonucleotide T δ E4 (from the human TCR δ gene enhancer; 18, 27) is included in the

Table 1. Oligonucleotides used in HIV-1 PCR-directed site specific mutagenesis

Oligonucleotide	DNA sequence						Corresponds to nt*
5'	CGAT	AAGCT	TGGAA	GGGCT	AATTC	ACT	−453 to −436
3'	GCT	TTCTA	GAGGC	TTAAG	CAGT		+71 to +60
site 1, 5'	CACT	GCATG	CGAGC	TTGCT	ACAA		−118 to −105
site 1, 3'	GCTC	GCATG	CAGCA	GTTCT	TGAA		−122 to −136
site 2, 5'	ATT	TGTCG	ACGTG	GCCCG	AGA		−165 to −153
site 2, 3'	CACG	TCGAC	AAATG	CTAGG	CGGC		−170 to −183
site 3, 5'	TCC	ACTCG	AGGTA	GAGAG	GCCA		−288 to −274
site 3, 3'	CTACC	TCGAG	TGGCT	CAACT	GGTA		−292 to −306
site 4, 5'	ATC	AGAGC	TCCAC	TGACC	TTTGG		−338 to −324
site 4, 3'	AGT	GGAGC	TCTGA	CCCCT	GG		−341 to −352
site 5, 5'	TTCCC	TGCAG	AGCAG	AACCTA	CAC		−372 to −306
site 5, 3'	TG	CTCTG	CAGGG	AAGTA	GCC		−376 to −388

*Nucleotide numbering shown is for the bold nucleotides in the center column, corresponding to the HIV-1 LTR sequence shown in Fig. 1A. Nucleotides shown in normal lettering (on the left; the 5' end in each case) were used to substitute restriction enzyme sites for hGATA-3 binding sites shown in Fig. 2A and 2B.

footprinting reactions, the protein:DNA complex at each of the six sites is disrupted (Figure 2A). These data show that hGATA-3 binds to six specific sites within the HIV-1 LTR transcriptional regulatory domain.

hGATA-3 *trans*-activates HIV-1 LTR-mediated transcription

We investigated the possibility that hGATA-3 might *trans*-activate HIV-1 LTR-mediated transcription by cotransfection experiments using a reporter gene whose activity was transcriptionally directed by the HIV-1 LTR. We initially examined the activity of this plasmid (and derivatives) after transfection into HeLa cells, where only low levels of hGATA-2, and no hGATA-1 or hGATA-3, is expressed. The HIV-1 LTR (nucleotides −376 to +80, Figure 1A) was subcloned into a promoterless reporter vector to generate HIVwt/CAT; this construct was cotransfected into HeLa cells with an activator plasmid constitutively expressing the hGATA-3 protein (RSV/hGATA-3; 18). As shown in Figure 3A, hGATA-3 indeed stimulates HIVwt/CAT expression by an average of seven-fold upon cotransfection into HeLa cells. Identical results were also obtained repeating the same experiments in QT6 cells, thereby demonstrating that the stimulatory effect detected in non-T cells is dependent only upon cotransfected hGATA-3 activator plasmid (31). These data show that the addition of hGATA-3 is capable of stimulating HIV-1 transcription in vertebrate cells which express little endogenous GATA factor.

To verify that the increase in expression of HIVwt/CAT was a direct effect of coexpressing functional hGATA-3 protein in the same cells, a deletion of amino acids 29 to 128 (which abrogates the *trans*-activation ability of the factor; 36) was introduced into the coding sequence of hGATA-3. As shown in Fig. 3A, the mutant hGATA-3 protein no longer *trans*-activates HIV transcription, although the mutant protein binds to a GATA oligonucleotide in EMSA (Fig. 2C) and stably accumulates in transfected cells (as confirmed by western blot analysis using an anti-hGATA-3 monoclonal antibody; 36). These data show that a fully functional hGATA-3 factor is required for *trans*-activation of HIV-1-directed expression *in vivo*.

To test whether the increase in expression was a direct effect of hGATA-3 binding to the identified GATA sites within the LTR of HIV-1 (Figure 2), a series of mutations were generated in the LTR where the GATA sequences (arbitrarily numbered 1 to 6; Figure 1) were individually changed into unique restriction enzyme recognition sites and then ligated into a single HIV-1

Figure 1. (A). Transcription factor binding sites in the HIV-1 LTR. Nucleotide sequence of the HIV-1 LTR (44) from the CAP site (nucleotide +1) extending 5' to the boundary of the U3 region is shown. Consensus transcription factor binding sites are underlined and the identity of the corresponding DNA binding proteins are shown either below or above the sequence (5, 6, 7, 9, 10, 11, 12, 14, 15, 16). The binding sites for hGATA-3 identified by *in vitro* footprinting (Figure 2A) are shown in outlined letters and are numbered 1 to 6 consecutively, beginning closest to the HIV-1 CAP site. The numbers at the left indicate the nucleotide number relative to the transcriptional start site. (B). GATA binding sites and their mutations. All six hGATA-3 binding sites and surrounding sequences are listed on the top line with the GATA sequence shown in bold. Each GATA site was mutated to a specific restriction enzyme site by PCR mutagenesis (underlined) as shown on the bottom line (Materials and Methods).

LTR (called noGATA; Figure 3C). DNase I footprint analysis confirmed that the mutations indeed eliminated hGATA-3 binding to the GATA sites identified in the footprinting assays (Fig. 2B).

The individual and multiple GATA binding site mutations were next functionally analyzed for their effect upon the ability of hGATA-3 to direct transcription from the HIV-1 LTR. Mutagenesis of individual GATA binding sites, including site 2 (within the NRE; 4), resulted in only a slight diminution in the transcriptional stimulation of the LTR by hGATA-3 (31). However, when all of the mutations were combined into a single

Figure 2. hGATA-3 binding to the HIV-1 wild type and mutated LTRs. (A). Footprinting of the wild type HIV-1 LTR U3 region. Lanes a–e and f–j show *in vitro* footprinting reactions of the coding and non-coding strands of the wild type HIV-1 LTR, respectively. Lanes a, f: no protein added; lanes b, g; c, h; and d, i contain 1 μ l, 3 μ l, or 10 μ l, respectively, of partially purified hGATA-3 (Materials and Methods). Lanes e and j contain 3 μ l of hGATA-3 plus 50 ng of unlabeled M α P GATA binding site oligonucleotide competitor (17, 29). GATA-3-specific footprints 1–6 are indicated by boxes shown to the left on the diagram, adjacent to each binding site for the coding strand, and on the right for the non-coding strand. (B). Mutation of the GATA sites eliminates hGATA-3 binding. Lanes a, b; c, d; e, f; g, h and i, j show the *in vitro* footprints obtained on the wild type HIV-1 LTR, or on LTRs reconstructed with mutated GATA footprints 1-, 3-, 4- or 5-, respectively. The first lane of each pair (a, c, e, g and i) represent footprints without added protein (as negative controls for each mutation), and the second (b, d, f, h and j) is with 1 μ l of partially purified hGATA-3 added to the reaction. The binding to mutated GATA site 2 was abolished when examined in gel shift assay (31). The numbers at left indicate GATA-3 footprints 1 to 6 as shown in Figure 1. (C). Transcriptional stimulation of HIV-1 transcription is due to exogenous hGATA-3 protein. QT6 cells were cotransfected with HIVwt/CAT and antisense or sense RSV/hGATA-3 DNA or mutant RSV/hGATA-3 d29-128 (Materials and methods; 31). The cells were then split and plated on two separate dishes; one set was used to assay HIV-1 LTR-directed transcription, while the other was used to prepare cellular extracts for EMSA (Materials and Methods). The probe is a radiolabeled TdE4 oligonucleotide containing two GATA binding sites (18). The hGATA-3 shifted bands are indicated at the left by arrows. Lane 1 is labeled oligonucleotide alone, while lanes 5 and 6 contain an additional 100 ng of unlabeled TdE4 oligonucleotide. Extracts were prepared from QT6 cells transfected with RSV/hGATA-3 (s; lanes 2, 5), antisense RSV/hGATA-3 (as; lane 4) or RSV/hGATA-3 d29-128 (lanes 3, 6).

plasmid (noGATA/CAT; Figure 3C) the ability of the LTR to be *trans*-activated by cotransfected hGATA-3 was significantly reduced. Taken together, these data demonstrate that hGATA-3 stimulates transcription by binding to the GATA sites within the HIV-1 LTR U3 regulatory region.

hGATA-3 binding is required for maximal expression from the HIV-1 LTR in both quiescent and induced T lymphocytes

We next examined the role of this T cell-restricted transcription factor in regulating HIV-1 expression in its natural environment, *i.e.* within T lymphocytes. The human T cell line Jurkat (TCR

$\alpha\beta^+$ CD4 $^+$), which expresses abundant hGATA-3 (18), was transfected with the HIVwt/CAT and the noGATA/CAT (Figure 3C) reporter plasmids. As do circulating T cells, Jurkat cells require two external stimuli to synthesize maximal amounts of lymphokine (37), and induction of T lymphocytes has been shown to result in increased levels of hGATA-3 transcription (38). After transfection of the various HIV/CAT plasmids, the cells were incubated for a total of 44 hours, including treatment with PMA and PHA for the final 20 hours. Transfection of either HIVwt/CAT or noGATA/CAT into Jurkat cells reveals a 10-fold induction of transcriptional activity when comparing stimulated

to untreated T cells (Figure 3B), even though the overall expression is significantly reduced in both resting or stimulated cells when noGATA/CAT is compared to HIVwt/CAT expression. Thus hGATA-3 exerts its effect(s) in activating the basal transcriptional apparatus through binding to the GATA sites within the LTR in T cells in a stimulation-independent manner, rather than through the same PHA + PMA stimulatory pathway of activation which induces NF- κ B (12).

DISCUSSION

We show here that the T lymphocyte transcription factor hGATA-3 binds *in vitro* with high specificity to six sites within the LTR of HIV-1 (Figure 2), and that the binding of hGATA-3 is required for a 7-fold increase in HIV-1 LTR-mediated transcriptional activation in non-lymphoid cells (Figure 3A). A mutated hGATA-3 factor, missing the transcriptional activation domain of the protein but which still binds to the GATA sites (Figure 2C), fails to *trans*-activate HIV expression. The reduction in HIV-1 transcription seen upon transfection of a mutated HIV-1 LTR (bearing mutations in all of the GATA binding sites) after transfection into T lymphocyte cells (Figure 3B) strongly supports the initial hypothesis that hGATA-3 is one of the cellular factors mediating viral LTR-directed transcription.

Mutation of all of the GATA binding sites did not fully abolish the transcriptional activity of the HIV-1 LTR in Jurkat cells. The U3 transcriptional regulatory domain of HIV-1 harbors binding sites for a large number of different transcription factors, many of which have been shown to bind (5, 6, 7, 10, 11, 12, 14, 15, 16), and several of which have been shown to exert direct transcriptional effects on this transcription unit (5, 6, 9, 10, 11, 12, 14). It could therefore be inferred that hGATA-3 acts in synergy with other cellular factors to stimulate HIV-1 transcription. Deletion of the GATA binding sites, as well as other transcription factor binding sites, might cause a more severe defect in transcription than mutation of the binding of any of these individual sites.

A previous analysis of the element within the HIV-1 LTR bearing homology to the IL-2 NRE identified a relatively abundant T cell-restricted binding factor of 50 kD, and it has previously been shown that the molecular weight of hGATA-3 on SDS gels is approximately 50 kD (18). It is possible that the protein bound by this presumptive regulatory element may be due to overlap of those sequences with GATA site 2 of the LTR (Fig. 1A; 6). If the binding protein identified in that study is hGATA-3, these results taken together suggest that hGATA-3 might function as a negative regulatory factor when placed in context with other regulatory proteins within the IL-2 NRE regulatory module, and thus hGATA-3 could have the property of being able to act as either a context-dependent positive (18, 27) or negative (39) transcription factor. However, we should be careful to point out that mutation of GATA sites 2 or 3 alone (Fig. 1B) display only slight alteration in phenotypic effect when these mutants are transfected into HeLa or Jurkat cells (31), mutations which should have theoretically disrupted cooperative interactions with any other protein bound nearby (e.g. those interacting with a presumptive IL-2 NRE-associated protein or with *c-myc*, respectively).

It has previously been shown that the presence of one of the two NF- κ B binding sites, one of the three Sp1 binding sites or the CAP-site proximal LBP-1 binding site is required for induction or repression, respectively, of HIV-1 transcription (5,

Figure 3. hGATA-3 regulation of HIV-1 transcription in transfected HeLa and Jurkat cells. (A) hGATA-3 *trans*-activates HIV-1 LTR-driven transcription in HeLa cells. Transfections were performed using either the HIVwt/CAT or noGATA/CAT plasmids with sense (s) hGATA-3, hGATA d29-128, or antisense (as) hGATA-3 plasmids as indicated to the left. Cell lysates were prepared and the CAT assays were quantified by determining the amount of acetylated [14 C] chloramphenicol produced in enzymatic assay; the degree of conversion was quantitated on a Molecular Dynamics PhosphorImager. The results shown are calculated relative to the conversion by cotransfection with antisense hGATA-3, and represent the average of four independent experiments. (B) hGATA-3-binding sites are required for *trans*-activation of the HIV-1 LTR in Jurkat cells. Jurkat cells were transfected with the HIV-1/CAT plasmids described below. After transfection, the extracts (normalized for equal amounts of luciferase activity) were used in CAT assays as above. CAT assays were quantified by PhosphorImager analysis, and the results shown are the average of three independent experiments using uninduced HIVwt/CAT transfection conversion percentage normalized as 1. The open bars represent the transfection results of cells stimulated by PMA + PHA and the shaded bar are those in the absence of induction. (C) HIV-1 LTR wild type and mutations. Line 1: the DNA sequence of the HIV-1 LTR, nucleotides -376 to +80. The noGATA construct (line 2) represents a plasmid in which each of the GATA binding sites was mutated by PCR (black boxes) into unique restriction enzymes sites (Figure 1B). Since deletion of GATA site 6 does not show a functional difference from the wild type HIV-1 LTR in transfection analysis (4,31), the noGATA construct contains only the LTR sequences from -376 to +80. All of the mutation LTRs were then subcloned 5' to a promoterless chloramphenicol acetyltransferase (CAT) gene (pCATbasic; Promega) and all of the constructs were confirmed by DNA sequencing.

10, 12). Mutation of individual GATA binding sites resulted in only very small quantitative changes in HIV-1-LTR mediated transcription; only by mutation of all of the GATA sites within the LTR do we see a significant reduction in the HIV-1 LTR-mediated gene expression, and that cumulative effect is smaller (by 2- to 3-fold) than the quite dramatic effect, for example, of mutation of the two NF- κ B sites. However, the data clearly demonstrate that the GATA binding sites are required for maximal expression of HIV-1 transcription. Furthermore, the GATA site mutations decrease activity in both stimulated and unstimulated T cells, but do not affect the relative inducibility of viral transcription through the PHA + PMA stimulatory pathway. Taken together, these results suggest that the interaction

of hGATA-3 with the basal transcriptional apparatus is cumulative and independent of phorbol ester or general mitogenic stimulation.

It has been shown that the binding of the CD4 receptor by the HIV envelope glycoprotein gp120 may be an early step in determining the cell type selectivity of the virus (40, 41). After HIV enters a cell, a continuation in this selectivity of tissue-specific expression might logically be assumed to be governed by further cell type-restricted mechanisms, including the involvement of tissue-restricted transcriptional regulatory proteins. The findings presented here that hGATA-3 is required for transcriptional activation of the HIV-1 LTR in T cells provides direct evidence for a T lymphocyte-restricted transcription factor which modulates transcription of the virus in which the tropism of infection is partially consistent with the documented expression of a tissue-restricted transcription factor. However, HIV replicates well in cell types which do not express GATA-3. Among the known members of this family, GATA-2 is expressed far more broadly than the other family members (17), and GATA-2 and GATA-3 have been shown to bind to the same subset of consensus DNA sequences in vitro (46). HIV-1 might also be regulated by other GATA factors in specific myeloid (42, 43) or neuronal (18, 20) cell lineages, other cell types in which HIV-1 is known to exert cytopathic effects (1) and which also express other GATA factors (17), is thus a subject of considerable interest, but awaits further detailed analysis.

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