Inhibition of Human Immunodeficiency Virus Type 1 Replication by a Tat-Activated, Transduced Interferon Gene: Targeted Expression to Human Immunodeficiency Virus Type 1-Infected Cells

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We have examined the feasibility of using interferon (IFN) gene transfer as a novel approach to anti-human immunodeficiency virus type 1 (HIV-1) therapy in this study. To limit expression of a transduced HIV-1 long terminal repeat (LTR)-IFNA2 (the new approved nomenclature for IFN genes is used throughout this article) hybrid gene to the HIV-1-infected cells, HIV-1 LTR was modified. Deletion of the NF- κ B elements of the HIV-1 LTR significantly inhibited Tat-mediated transactivation in T-cell lines, as well as in a monocyte line, U937. Replacement of the NF- κ B elements in the HIV-1 LTR by a DNA fragment derived from the 5'-flanking region of IFN-stimulated gene 15 (ISG15), containing the IFN-stimulated response element, partially restored Tat-mediated activation of LTR in T cells as well as in monocytes. Insertion of this chimeric promoter (ISG15 LTR) upstream of the human IFNA2 gene directed high levels of IFN synthesis in Tat-expressing cells, while this promoter was not responsive to tumor necrosis factor alpha-mediated activation. ISG15-LTR-IFN hybrid gene inserted into the retrovirus vector was transduced into Jurkat and U937 cells. Selected transfected clones produced low levels of IFN A (IFNA) constitutively, and their abilities to express interleukin-2 and interleukin-2 receptor upon stimulation with phytohemagglutinin and phorbol myristate acetate were retained. Enhancement of IFNA synthesis observed upon HIV-1 infection resulted in significant inhibition of HIV-1 replication for a period of at least 30 days. Virus isolated from IFNA-producing cells was able to replicate in the U937 cells but did not replicate efficiently in U937 cells transduced with the IFNA gene. These results suggest that targeting IFN synthesis to HIV-1-infected cells is an attainable goal and that autocrine IFN synthesis results in a long-lasting and permanent suppression of HIV-1 replication.

Human interferon A (IFNA) inhibits replication of retroviruses, including human immunodeficiency virus type 1 (HIV-1), an etiological agent of AIDS (11, 17, 21, 26, 41, 55). Although HIV-1 is sensitive to IFN-mediated inhibition, HIV-1 infection does not induce expression of IFNA and IFNB genes in T cells or monocytes. The efficacy of IFNA in the treatment of HIV-1 infection and AIDS-associated Kaposi's sarcoma has been demonstrated in clinical trials (10, 27, 45). Nevertheless, the clinical use of IFN has been limited by frequent side effects induced by the administration of high levels of exogenous IFN (7). Furthermore, effective inhibition of HIV-1 replication in vivo may require continuous administration of IFN, since the inhibitory effect in vitro was reversed upon IFN removal (22, 42, 62). Consequently, for an optimal IFN effect and effective therapy or prophylaxis of AIDS, an improved method of IFN delivery is desirable. Targeting IFN synthesis to a focus of infection by a transduced IFN gene(s) may provide the most effective approach.

In our previous study (4), we showed that HIV-1 replication was inhibited in human T cells containing the integrated HIV-1 long terminal repeat (LTR)-IFNA2 (the new approved no-

menclature for IFN genes is used throughout this article [10a]) hybrid gene. IFN synthesis was increased by about 10 to 100 times when these cells were transfected with Tat-expressing plasmid. However, virus-encoded Tat protein is not the only factor that can activate transcription of HIV-1 LTR and mitogenic stimuli and cytokines, or infection with viruses of herpes group activates HIV-1 LTR in a Tat-independent fashion through the enhancer elements present in the HIV-1 LTR (12, 18, 20, 23, 33, 43, 60). Thus, activation through these enhancers may lead to nonspecific activation of a LTR-IFN hybrid gene in the absence of HIV-1 infection.

Among enhancer elements present in the LTR region, the two κ B motifs were shown to play a crucial role in activating expression of HIV-1 provirus in latently infected T cells (35). Transcriptional activation by the NF- κ B enhancer is mediated by binding of a NF- κ B heterodimer composed of 50-kDa (p50) and 65-kDa (p65) subunits (2, 25, 59). In most of the cells, the classic NF- κ B (p50-p65) is present in an inactive form in cytoplasm where it associates with the inhibitory protein I κ B (MAD3) (1, 63). Activation of NF- κ B, which involves dissociation of p50/p65 heterodimer from I κ B and subsequent transport to the nucleus, can be triggered by a variety of extracellular stimuli, including cytokines such as tumor necrosis factor alpha (TNF- α) and interleukin-1 (IL-1) (38), double-stranded RNA (29), herpes simplex virus type 1 infection (18, 61), viral transactivators Tax and hepatitis B virus X protein (3, 58), T-cell mitogens (54), bacterial lipopolysaccharide (54), UV light (57), and hydrogen peroxide (53).

It is well established that IFNA exerts its effects by binding

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to a specific cell surface receptor and subsequently enhancing the transcriptional activities of a specific set of IFN-stimulated genes (16, 28). The coordinate transcriptional control of these genes depends on the presence of a consensus enhancer sequence, the IFN-stimulated response element (ISRE) (31). Since our goal has been to obtain prolonged high concentrations of IFNA at the site of infection, we wished to determine whether IFN production from a transduced gene could be augmented through a putative feedback mechanism. Using a HIV-1 LTR-chloramphenicol acetyltransferase (CAT) plasmid that had a κ B enhancer deleted, we examined the responsiveness of a plasmid with this κ B-deleted LTR to Tat in various cell types that can be infected with HIV-1. We also tested the possibility of maintaining high levels of Tat-mediated IFN expression by substituting the two κ B motifs in HIV-1 LTR with either ISRE or a segment of the promoter region of the ISG15 gene (IFN-stimulated gene 15) containing an ISRE (28). Our results indicate that the deletion of the two NF- κ B enhancer elements significantly decreased the efficiency of Tat-mediated transactivation of the HIV-1 LTR in both T cells and monocytes. Replacement of NF- κ B enhancers by the promoter region of the ISG15 gene increased Tat-mediated transactivation in both types of cells, while this promoter was not activated by TNF- α . HIV-1 infection of T cells and U937 cells containing the transactivable IFNA gene leads to stimulation of IFNA synthesis and long-lasting inhibition of HIV-1 replication.

MATERIALS AND METHODS

Cell cultures. HeLa, Vero, and bovine embryonic trachea cells (ATCC CCL-44) were maintained in Dulbecco modified Eagle medium (Life Technologies, Inc. Grand Island, N.Y.) with 10% fetal bovine serum, 2 mM glutamine, and 50 μ g of gentamicin per ml. A3.01, CEM-174, Jurkat, and U937 cells were maintained in RPMI 1640 medium (Life Technologies) at a density of 3×10^5 cells per ml as previously described (55).

Plasmid constructions. Cloning of various restriction fragments was performed as described by Sambrook et al. (51). The ISG15-CAT hybrid plasmid was constructed by inserting the *EcoRI*-*Bam*HI fragment, which contains -366 to +44 of the ISG15 promoter from plasmid NcoISG15-E1B (46), between the *Pst*I and *Bam*HI sites of pBLCAT-3 (32). The (ISRE)₃(-119) HIV-1 LTR-CAT plasmid was constructed by subcloning an *EcoRI* fragment, containing three copies of ISRE of ISG15 from ISG15-TK-3 plasmid (47), into the *Xho*I site of (-119) HIV-1 LTR-CAT (48). ISG15(-83) HIV-1 LTR-CAT plasmid was constructed by subcloning a *Hind*III-*Dde*I fragment containing -366 to -36 of the ISG15 promoter between the *Hind*III and *Pst*I sites of (-83) HIV-1 LTR-CAT (56). Retrovirus vectors containing the modified HIV-1 LTR-IFNA2 hybrid gene were constructed by cloning the IFNA2 gene (4) between the *Hind*III and *Bam*HI sites of pBluescript SK plasmid (Stratagene, La Jolla, Calif.). The *Hind*III-*Kpn*I fragments containing either the ISG15(-83) HIV-1 LTR or HIV-1 LTR (-83 to +80) were then subcloned into the *Cl*aI site of this plasmid. Finally, the *Xho*I-*Xba*I fragment containing the modified HIV-1 LTR-IFNA2 hybrid gene from the plasmid described above were subcloned into the *Bam*HI site of retrovirus vector pJenH⁻ (13).

Transfection and induction of expression in a transient transfection assay. HeLa and Vero cells were transfected by calcium phosphate coprecipitation as previously described (6). Briefly, cells were seeded at a density of 5×10^5 cells per 60-mm-diameter plate, and 24 h later, 2 μ g of reporter CAT or IFNA plasmid and 1 μ g of Tat-encoding plasmid (pIII_{extat}III) were added as calcium phosphate precipitate. After 5 h of incubation, cells were treated with 15% glycerol for 1 min, washed twice with phosphate-buffered saline, and incubated for an additional 48 h. CAT extracts were then prepared as previously described (19). The A3.01, CEM-174, Jurkat, and U937 cells were transfected with similar amounts of plasmids by DEAE-dextran coprecipitation as described previously (24). Briefly, 5×10^6 cells were washed and incubated with plasmid DNA in 1 ml of DEAE-dextran (100 μ g/ml; Pharmacia, Piscataway, N.J.) in RPMI 1640 medium for 10 min at 37°C. The cells were then washed, refed with medium, and incubated for 48 h before harvest for the CAT assay.

To examine the effect of IFNA, transfected cells were divided into duplicate plates after DEAE-dextran was washed off, and 16 h later, human recombinant IFNA (Hoffman-LaRoche Inc., Nutley, N.J.) was added at a concentration of 1,000 U/ml to one of the plates. Cells were processed for the CAT assay 24 h after the addition of IFNA. To examine the effect of IFNA on Tat-mediated transactivation, CEM-174 or U937 cells were cotransfected with reporter CAT plasmids and pIII_{extat}III (1 μ g). The reproducibility of the transfection experi-

ments was ascertained by repeating each experiment at least twice. Comparable results were obtained in all instances. Permanently transfected cell lines were established by electroporation of the retrovirus vectors containing hybrid IFNA genes and selection of the transduced cells by growth in G418. The selected cells were cloned by limiting dilutions and allowed to grow into the cultures.

RNA isolation and analysis. Total RNA was prepared from HeLa cells, collected 24 h after transfection, and U937 cells, 14 days after infection with HIV-1, by the guanidinium thiocyanate method (8). Northern (RNA) blot hybridization was performed as previously described (44), using a CAT riboprobe. To examine IFN-induced expression of ISG15, cells were treated with IFNA (1,000 U/ml) for 3 h, and total RNA was isolated by the acid phenol-guanidinium thiocyanate procedure (9) and analyzed by Northern blot hybridization with an ISG15 riboprobe.

IFN assays. The media were collected from Vero, U937, or Jurkat cells 48 h after transfection. The media from the permanently transfected Jurkat and U937 cells before or after HIV-1 infection were collected as indicated. Biologically active IFN was assayed in duplicate by inhibition of the cytopathic effect of vesicular stomatitis virus on bovine embryonic trachea cells (15). The level of IFN antiviral activity in each sample was quantitated by comparison with standards of human recombinant IFNA2 (Hoffman-LaRoche).

HIV-1 infection. The HIV-1 pNL4-3 strain obtained from M. Martin was used in all experiments. Virus stock was obtained from activated peripheral blood mononuclear cells (PMBC) and titrated on C8166 cells, and unless otherwise stated, all infections were done with a multiplicity of infection (MOI) of 0.01. Jurkat and U937 cells and the permanently transfected clones were plated at 10^6 cells per ml in RPMI 1640 medium with 10% fetal calf serum and infected with the pNL4-3 strain of HIV-1. Every third day, 50% of culture medium was removed and replaced with an equal volume of fresh medium. Virus in the collected medium was analyzed by a reverse transcriptase (RT) assay as described previously (55).

Stimulation of IL-2 and IL-2R gene expression. Jurkat cells or JISGA3A clone cells were stimulated with phytohemagglutinin (PHA) (5 μ g/ml) and phorbol myristate acetate (PMA) (50 μ g/ml) for 16 h; cells were then washed and harvested, and total RNA (10 μ g) was analyzed by Northern blot hybridization with IL-2 DNA probe (pCDHuIL-2) and IL-2 receptor B (IL-2RB)-specific riboprobes.

RESULTS

NF- κ B-binding sites are required for efficient Tat activation in human T cells and monocytes. Several earlier studies had addressed the question of the importance of NF- κ B elements of HIV-1 LTR in Tat-mediated transactivation with somewhat different results (36, 56). Therefore, we initially tested whether the NF- κ B-binding sites could be deleted without affecting the magnitude of Tat activation of HIV-1 LTR. Several human T-cell lines (A3.01, CEM-174, and Jurkat) and one promonocytic cell line (U937) were chosen as hosts, and expression of the plasmids containing HIV-1 LTR with (-119) or without (-83) (κ B HIV-1 LTR) NF- κ B-binding sites was examined in the presence or absence of a Tat-expressing plasmid (pIII_{extat}III). As shown in Fig. 1, expression of a κ B-deleted mutant [(-83) HIV-1 LTR] was not efficiently activated by Tat in any of the three T-cell lines tested (lanes 4, 8, and 12) when measured under conditions of linear response. A low degree of activation of the κ B-deleted mutant [(-83) HIV-1 LTR] could be achieved when 10-fold-higher levels of Tat-expressing plasmid were used for transactivation; however, these conditions were far out of the linear response and therefore, not meaningful for our comparative analyses. Tat-mediated activation of the (-83) HIV-1 LTR was observed in U937 cells (lane 16), but the levels of CAT activity were at least fivefold lower than those expressed by a plasmid containing the intact NF- κ B-binding sites (lane 14).

Since earlier studies have shown that the NF- κ B-binding sites are not required for Tat activation in either Jurkat or HeLa cells (56), we examined the reasons for this discrepancy. In contrast to T cells and monocytes, we observed significant Tat activation from the κ B-deleted mutant in HeLa cells (lane 20). However, when analyzed under conditions of a linear response, a dramatic difference in Tat activation of (-119) HIV-1 LTR and (-83) HIV-1 LTR was found, even in HeLa cells (lanes 21 and 22). These data confirm the previous

FIG. 1. Efficient activation of HIV-1 LTR by Tat requires the presence of κ B motifs. (–119) HIV-1 LTR and (–83) HIV-1 LTR-CAT (shown schematically at the top of the figure) were transfected with (+) or without (–) pIII κ III (Tat) into T cells and monocyte lines. The CAT assay was performed 48 h after transfection by using 40 μ g of cell extract in a 1-h reaction at 37°C, except for the assays shown in lanes 21 and 22 in which 20 μ g of cytosol was used and incubation was for 15 min. In A3.01 cells transfected with (–119) HIV-1 LTR-CAT (–119), the conversion was in the range of 1.5 to 2.4% in the absence of Tat and 65 to 75% in the presence of Tat, while in cells transfected with (–83) HIV-1 LTR-CAT (–83), the values for conversion were 1.2 and 2.1%, respectively; the exception was U937 cells in which the conversion of this plasmid was about 6% in the presence of Tat. In the gel in the lower right-hand of the figure, total RNA (15 μ g) prepared from HeLa cells at 24 h posttransfection was analyzed by Northern blot hybridization with the CAT antisense riboprobes.

observation that in HeLa cells, the NF- κ B sites are not required for transactivation. These data also clearly show that the magnitude of Tat transactivation is lower in the absence of NF- κ B-binding factors and that the effect of Tat and NF- κ B is more than additive.

To confirm that CAT activity is a valid measure of steady-state CAT mRNA levels, we analyzed the levels of CAT RNA in HeLa cells 24 h after transfection. As shown in the gel in Fig. 1, the (–83) HIV-1 LTR-CAT plasmid was transactivated by Tat (lane 4), but the relative levels of CAT mRNA were much lower than those encoded by the wild-type HIV-1 LTR-CAT plasmid (lane 2). To rule out the possibility that the observed difference in CAT activity might have resulted from different transfection efficiencies, the amounts of plasmid in the cytosol from the transfected cells were determined by dot blot analysis (34). No significant differences in the amount of plasmid DNA were found between each transfection (data not shown). These results indicate that the NF- κ B-binding sites of HIV-1 LTR may not be essential for Tat activation in HeLa or U937 cells, but these *cis* elements appear to be much more important for Tat activation in the human T-cell lines we tested. It should be pointed out that although others (49) have shown that HIV-1 provirus which had NF- κ B sites deleted was able to replicate in PBMC, its replication rate was much slower than that of the wild type.

Expression of ISG15 in human T and promonocytic cells can be induced by IFNA. To compensate for the decrease of

promoter activity of HIV-1 LTR caused by removal of the NF- κ B-binding sites, we examined whether insertion of a well-defined IFN response element (ISRE) from the ISG15 promoter region (46), upstream of the HIV-1 LTR, could enhance the promoter activity of HIV-1 LTR. Since most of the early studies showed IFNA-mediated induction of ISG15 gene in HeLa or other human fibroblast cell lines (16, 31, 46), we examined whether the ISG15 gene can also be induced in IFNA-treated human T cells (A3.01, CEM-174, and Jurkat) and monocytic U937 cells. The results of Northern blot analysis (Fig. 2) showed inducible expression of the ISG15 gene in all cell lines except A3.01 cells, which are rather insensitive to the IFNA effect (data not shown). To verify that the ISRE present in the promoter region of ISG15 at positions –111 to –94 is sufficient to confer an IFN response in cells other than fibroblasts, the promoter regulatory region (–366 to +44) of the ISG15 gene was inserted upstream of the CAT reporter gene. This construct was transfected into HeLa, CEM-174, or U937 cells, and 16 h after transfection, the cells were treated with IFNA for 24 h and CAT activity was measured. The ISG15 promoter conferred IFN response to the reporter gene in all three cell lines tested (Fig. 2A, lanes 2, 4, and 6). A plasmid containing three copies of ISRE 5' of (–119) HIV-1 LTR-CAT was also induced in U937 (Fig. 2B, lane 8) and CEM-174 cells (data not shown) although less efficiently. These results indicate that while ISRE alone can function in human T cells and monocytes, additional *cis*

FIG. 2. IFN induces expression of the endogenous ISG15 gene and transfected ISG15 CAT plasmids in human T cells and macrophages. (A) Northern blot analysis of the ISG15 mRNA in HeLa, T cells, and monocytes treated with human IFNA2. The arrow indicates the position of ISG15 mRNA. (B) Transient transfection assay with plasmids (shown schematically) containing either the ISG15 promoter (ISG 15 PROM) or the (-119) HIV-1 LTR promoter with three copies of ISREs inserted 5' of the CAT gene. Lanes 1 through 6, cells transfected with ISG15 CAT; lanes 7 and 8, cells transfected with (-119) HIV-1 LTR-CAT. The percentages of conversion of the uninduced and IFNA-induced ISRE-HIV-1 LTR-CAT plasmid were 4 to 8 and 55 to 60, respectively.

element(s) in the ISG15 promoter region may contribute to the magnitude of induction.

Activity of ISG15-HIV-1 LTR chimeric promoter is enhanced by Tat. Since a higher response to IFNA was observed when the ISG15 promoter, rather than multiple copies of ISRE, was linked upstream of the HIV-1 LTR, in the following studies, an ISG15 promoter (-366 to -36) lacking the TATA box inserted upstream of (-83) HIV-1 LTR-CAT was used. The inducibility of this plasmid by IFNA in the presence or absence of Tat was first examined in CEM-174 cells. Figure 3 shows that while transfected ISG15 (-83) HIV-1 LTR-CAT plasmid was not expressed constitutively in these cells (lane 5), treatment of transfected cells with exogenous IFNA2 resulted in an approximately 4-fold increase of CAT activity (lane 6), while Tat-induced expression of ISG15 HIV-1 LTR-CAT plasmid was about 10-fold higher than expression of (-83) HIV-1 LTR-CAT plasmid (lanes 4 and 7), suggesting that factors binding to the enhancer(s) in the ISG15 promoter region can cooperate with Tat protein. However, the addition of exogenous IFN did not increase significantly (twofold) CAT levels in the presence of Tat, indicating that the Tat-mediated transactivation of this promoter is much stronger than the stimulation of ISRE induced by ISGF-3 complex (30).

ISG15-HIV LTR-CAT was also transfected into U937 cells. In these cells, transactivation of the chimeric promoter by Tat was about 4- and 20-fold higher than the (-119) and (-83)

FIG. 3. Linkage of the 5'-flanking sequences of the ISG15 gene can partially restore Tat-mediated activation of (-83) HIV-1 LTR in CEM-174 cells. (A) ISG15 (-83) HIV-1 LTR-CAT plasmid is shown schematically. CEM-174 cells were transfected (+) with a given plasmid and induced (+) or not induced (-) with recombinant human IFNA (500 U/ml) 24 h after transfection as described in Materials and Methods. CAT activities were measured 40 h after transfection. (B) Activation of (-119) HIV-1 LTR and ISG15 (-83) HIV-1 LTR-CAT expression by TNF- α in U937 cells. U937 cells (10^7) were transfected with either (-119) HIV-1 LTR-CAT or ISG15 (-83) HIV-1 LTR-CAT as described in Materials and Methods and then divided into two plates. Twenty-four hours after transfection, recombinant human TNF- α (TNF-A) (100 U/ml; Genentech, South San Francisco, Calif.) was added to the medium while the other plate was used as a uninduced control. Incubation was continued for a further 20 h before the CAT assay was performed.

HIV-1 LTRs, respectively (Table 1). Treatment of transfected cells with IFNA did not greatly affect the Tat-mediated activation of (-119) HIV-1 LTR and (-83) HIV-1 LTR, while it slightly stimulated the expression of ISG15 (-83) HIV-1 LTR.

The activation of modified HIV-1 LTR by cytokines was also tested. In contrast to the (-119) HIV-1 LTR, the ISG15 (-83) HIV-1 LTR could not be activated by TNF- α (Fig. 3B) or IL-1 (data not shown), suggesting that the modified HIV-1 LTR is not sensitive to NF- κ B-mediated transactivation. Surprisingly, however, this promoter was still responsive to PMA-mediated stimulation, indicating that not only NF- κ B binding, but as shown in megakaryocytes (50), additional alternative pathways are involved in PMA-mediated stimulation.

Tat-induced IFN synthesis in cells transiently transfected with retrovirus vectors containing the (-83) HIV-1 LTR and ISG15-HIV-1 LTR hybrid genes. Since the ultimate goal of this study was to obtain a promoter that could express the IFN gene selectively in HIV-1-infected cells, we further tested

TABLE 1. Enhancement of Tat-mediated activation of the (–83) HIV-1 LTR in U937 cells by linkage of *cis* sequences of the ISG15 promoter^a

Reporter	Cotransfected plasmid	IFNA ^b	% Conversion
(–119) HIV-1 LTR	None	–	0.5
	None	+	0.6
	pIII <i>extat</i> III	–	86.8 ^c
	pIII <i>extat</i> III	+	53.8
(–83) HIV-1 LTR	None	–	0.3
	None	+	0.3
	pIII <i>extat</i> III	–	16.7
	pIII <i>extat</i> III	+	12.7
ISG15 (–83) HIV-1 LTR	None	–	0.6
	None	+	5.3
	pIII <i>extat</i> III	–	316.6 ^c
	pIII <i>extat</i> III	+	443.3 ^c

^a U937 cells were transfected and treated with human IFNA as described in Materials and Methods.

^b –, IFNA absent; +, IFNA present.

^c Reduced amounts of cell extract and reaction times were used for the CAT assay when acetylation of CAT was higher than 60%. All the values shown were normalized to a reaction time of 30 min with 50 µg of cytosol.

whether the ISG15–HIV-1 LTR hybrid promoter could be used to direct high levels of IFN synthesis in the presence of Tat proteins. We inserted the ISG15–HIV-1 LTR chimeric promoter upstream of the human IFNA2 gene, and this hybrid gene was then introduced into pLJen^h retrovirus vector which has a deletion in the enhancer region of its 3' LTR (13, 14). Similar vectors containing either the entire (–119) HIV-1

LTR or the (–83) HIV-1 LTR-IFNA hybrid gene were used as the positive or negative control, respectively. Synthesis of IFNA2 from these retrovirus vectors in the presence or absence of Tat protein was first analyzed in Vero cells, which contain a homozygous deletion of type 1 IFN genes (IFNA and IFNB), and therefore, any IFN synthesis in these cells is encoded by the transduced IFN gene (34). Culture medium was collected 48 h after transfection, and levels of biologically active IFNA were measured as described in Materials and Methods. As shown in Fig. 4, in the absence of Tat protein, the amount of IFNA produced from the vector containing the (–83) HIV-1 LTR-IFNA hybrid gene inserted in the opposite (–) orientation was twofold higher than the amount obtained from the vector carrying the hybrid gene inserted in the forward (+) orientation. In the presence of Tat, a 4-fold increase of IFNA synthesis was observed from the vector containing the forward-oriented hybrid gene, while more than a 20-fold increase of IFN synthesis was observed from the vector carrying the hybrid gene in the reverse orientation. Orientation-dependent activation of an inducible promoter in this retrovirus vector was also observed in our previous studies (4, 14) and is presumably due to the presence of an enhancer in the 3' adjacent simian virus 40 promoter directing the expression of the neomycin-resistant gene (14). Linkage of the 5'-flanking region of ISG15 gene to (–83) HIV-1 LTR resulted in a four- to fivefold increase of a constitutively produced IFNA from the retrovirus constructs, suggesting that the ISRE enhancer present in the linked ISG15 DNA fragment functions in Vero cells. When an ISG15-LTR-IFNA2 hybrid gene was inserted in the opposite orientation in this vector, a relative magnitude of transactivation (16-fold) was similar to that obtained from the (–83) HIV-1 LTR. However, when an ISG15-LTR-IFNA2 hybrid gene was inserted in the forward

FIG. 4. Activation of IFNA2 synthesis by Tat in Vero, U937, and Jurkat cells transfected with the retrovirus vectors containing the modified HIV-1 LTR-IFNA gene. The HIV-1 LTR-IFNA hybrid genes inserted into the pLJen^h vector (13) were transfected in the presence (+Tat) or absence (–Tat) of pIII*extat*III into Vero, U937, or Jurkat cells, and IFN levels in medium of transfected cells were measured 48 h after transfection as described in Materials and Methods. It should be noted that all the plasmids contain the *neo* gene under regulation of the simian virus 40 early promoter region inserted 3' of the IFN gene.

FIG. 5. Tat-inducible expression of IFNA2 mRNA in transfected cells. Cells were transfected with retrovirus vector in the presence and absence of pII β extatIII plasmid as described in the legend to Fig. 4, and total RNA (10 μ g) isolated from U937 cells 48 h after transfection was analyzed by Northern blot hybridization with human IFNA2 antisense riboprobes. Lanes 1 and 2, RNAs from cells transfected with vector III; lanes 3 and 4, RNAs from cells transfected with vector IV; lanes 5 and 6, RNAs from cells transfected with vector I. Vectors I, III, and IV are shown in Fig. 4. In lanes 2, 4, and 6, cells were cotransfected with pII β extatIII. The arrow indicates the position of IFNA2 mRNA.

orientation into this retrovirus vector, Tat-mediated expression of IFN gene was much higher (20-fold) in the presence than in the absence (5-fold) of a ISG15 fragment. These data indicate that the presence of heterologous enhancer (ISG15 or simian virus 40) 5' of (–83) HIV-1 LTR enhances Tat-mediated transactivation.

Production of IFNA from these retrovirus vectors was also analyzed in monocyte (U937) and T-cell (Jurkat) lines. In U937 cells, constitutive levels of IFN were very low (5 to 16 U) and were not increased by a linkage of the ISG15 DNA fragment to the (–83) HIV-1 LTR; however, the presence of ISG15 greatly facilitated Tat-activated synthesis of IFN, and about a 38-fold increase in IFN synthesis was observed. The high level of transactivation of this hybrid promoter was confirmed when the levels of IFNA mRNA were analyzed by Northern blot hybridization (Fig. 5). The relative levels of IFNA mRNA directed by the ISG15-LTR hybrid promoters in the presence of Tat were about 10-fold higher than the levels encoded by the (–83) HIV-1 LTR promoter (Fig. 5, compare lanes 2 and 4 with lane 6).

In contrast, in T cells, no constitutive IFNA synthesis could be detected in the absence of Tat and neither the (–83) HIV-1 LTR nor ISG15-HIV-1 LTR-IFNA hybrid were expressed constitutively and no IFN activity could be detected in the medium. Only in the presence of Tat protein were these promoters transcriptionally activated. Similarly, as in U937 cells, plasmids containing the (–83) HIV-1 LTR or ISG15-HIV-1 LTR-IFNA hybrid gene inserted in the opposite orientation were induced by Tat more efficiently than when the hybrid gene was inserted in the forward orientation. These results indicate that constitutive expression of the (–83) HIV-1 LTR and ISG15-HIV-1 LTR is determined by the host factors and that in T cells, transient expression of a modified HIV-1 LTR is strictly dependent on the presence of Tat. In contrast, the HIV-1 LTR promoter containing the enhancer region (–451 HIV LTR) was expressed constitutively in Jurkat cells even in the absence of Tat and relatively high levels (100 U) of IFNA could be detected in the medium.

Yields of the recombinant virus are inhibited by IFNA synthesized in the packaging line. To be able to transduce effectively the (–83) HIV-1 LTR or ISG15-HIV-1 LTR-IFNA hybrid genes into T cells and monocytes, we have attempted to package these hybrid genes into amphotropic virus pseudotypes. Retrovirus vectors containing various HIV-1 LTR hybrid genes (Fig. 5A) were transfected into PA317 cells, neomycin-resistant clones were selected, and the levels of a recombinant virus in medium were analyzed by a RT assay and by the titration of the recombinant virus on NIH 3T3 cells and counting the neomycin-resistant colonies. While the levels of

RT in the medium of transfected cells were about 10- to 15-fold higher than in the untransfected cells, a titration on NIH 3T3 cells showed that only about 100 to 500 particles per ml are present in the medium. In contrast, PA317 cells transfected with the vector alone produced about 5×10^4 to 5×10^5 neomycin-resistant particles per ml. Transfected cells were, therefore, cloned, and the individual clones were tested for the production of recombinant virus, but no clone that produced more than 1,000 particles per ml was identified. However, all clones tested produced low levels (5 to 10 U/ml) of human IFNA2 constitutively (data not shown).

The low packaging efficiency could be due to the inability of this particular construct to assemble efficiently or a consequence of the low constitutive IFNA synthesis in packaging cells. We have previously shown that assembly of murine retrovirus particles is impaired in the presence of IFN (40). To be able to distinguish between these two possibilities, we transfected into PA317 cells another retrovirus construct, containing the (–119) HIV-1 LTR-IFNA hybrid gene inserted into the pLNL6 vector. In contrast to a pLJenh⁺ vector used previously (14), this vector has unmodified 3' LTR, and the recombinant gene is inserted 3' of the neomycin gene and is therefore, effectively expressed from a spliced mRNA (5). The neomycin-resistant colonies were selected, and the levels of virus and IFNA2 in medium of the individual clones were tested. The results in Fig. 6 suggest an inverted relationship between IFNA2 production and levels of recombinant virions in the medium. The highest yields of a recombinant virus were observed in clones that did not synthesize IFNA2 efficiently (less than 20 U), while a majority of the clones that produced about 50 to 300 U of IFNA constitutively yield low levels of virus particles. These data indicate that constitutive IFNA2 synthesis in the packaging line interferes with the assembly of the recombinant virus.

IFN synthesis in stably transfected T cells and monocytes and its effect on immune functions. Because of an inefficient packaging of the recombinant retrovirus vectors in the amphotropic cells, retrovirus vectors containing the (–83) HIV-1 LTR-IFNA2 and ISG15-HIV-1 LTR-IFNA2 hybrid plasmids (Fig. 4) were transduced into Jurkat cells and U937 cells by electroporation, and neomycin-resistant clones were selected and tested for spontaneous production of IFNA2. While the pool of transduced clones produced about 50 U of IFNA per ml constitutively, the individually selected transduced U937 clones produced constitutively variable levels of IFNA (Table 2). A majority of (–83) HIV-1 LTR-IFNA2-transduced clones (60 to 70%) produced low levels (5 to 50 U/ml) of spontaneous IFNA, while a small percentage (5 to 20%) of U937 clones synthesized more than 100 U of IFNA per ml. Similarly, 70 to 76% of U937 clones containing integrated ISG15-HIV-1 LTR-IFNA hybrid gene synthesized low levels of IFNA (0 to 50 U/ml), and about 20 to 30% of clones synthesized more than 100 U/ml. These differences in expression among individual clones may be due to the number of integrated copies, as well as the position of the integration site. However, the levels of constitutively produced IFNA were generally higher in U937 cells than in Jurkat cells (data not shown). In contrast to the transient expression assays, where the expression of (–83) HIV-1 LTR-IFNA and ISG15-HIV-1 LTR-IFNA plasmids in T cells was seen only in the presence of Tat, low levels of spontaneous IFNA synthesis (20 to 40 U/ml) could be detected in the pool of transfected clones and in a majority of permanently transduced T-cell clones tested (data not shown).

In order to determine whether constitutive IFNA synthesis interferes with the T-cell immune functions, we analyzed and

FIG. 6. Relationship between production of recombinant virus and IFNA2 synthesis in PA317 cells transfected with pLNL6-A-9 plasmid. The packing line PA317 was transfected with recombinant vector pLNL6, containing the IFNA2 cDNA under the regulation of (–119) HIV-1 LTR. G418-resistant clones were selected and grown to confluence in 24-well plates. The levels of biologically active IFNA2 (A) or recombinant virus (B) in the medium of individual clones were determined as described in Materials and Methods. The relationship between IFNA2 synthesis and RT activity (C) is shown.

compared the expression of IL-2 and IL-2R genes in Jurkat cells and JISG3-A cloned cells 16 h after stimulation with PHA and PMA. It can be seen in Fig. 7 that while no IL-2 and IL-2R mRNAs can be detected in unstimulated cells, the relative levels of both of these mRNAs are comparable in stimulated Jurkat and JISG3-A cells. These results show that constitutive

FIG. 7. Stimulation of expression of IL-2 and IL-2R β genes by PMA and PHA in Jurkat and JISG3-A cells. Total RNA (10 μ g/lane) isolated from cells (10^7) stimulated with PMA (50 ng/ml) and PHA (5 μ g/ml) for 16 h was analyzed by Northern blot hybridization with 32 P-labeled IL-2 DNA probe (pcDHuIL-2) and IL-2R β DNA probe as described previously (44).

IFNA synthesis does not alter the ability of T cells to respond to mitogenic stimuli.

HIV-1 replication is inhibited in Jurkat and U937 cells containing integrated HIV-1 LTR-IFNA2 hybrid plasmids. Replication of HIV-1 was analyzed in a clone of Jurkat cells permanently transfected with the retrovirus vector containing the recombinant ISG15-HIV-1 LTR-IFNA2 plasmids. The selected clone (JISG3-A), produced low levels of IFNA2 constitutively, but IFNA2 synthesis could be greatly enhanced upon transfection with the Tat-expressing plasmid (data not shown). Kinetics of HIV-1 replication in Jurkat cells and in a clone (JISG3-A) of Jurkat cells transfected with the ISG15-LTR-IFNA2 plasmid (construct IV in Fig. 5A) are compared in Fig. 8A. It can be seen that while the levels of RT activity in the medium of infected Jurkat cells gradually increased, reaching maximum at 10 days postinfection, in JISG3-A cells HIV-1 replication was permanently suppressed. Furthermore, in contrast to infected Jurkat cells (which, by 10 days postinfection, started to show cell fusion; by 2 weeks postinfection, more than 50% of the cells were dead), no cytopathic effect was observed in the infected JISG3-A clone, and the infected cells were viable without any detectable cytopathic effect for a month postinfection, when the experiment was terminated (data not shown).

The levels of IFNA2 synthesized in infected and uninfected JISG3-A cells were then analyzed. The levels of IFNA2 in uninfected cells during the first 4 days were negligible (less than 20 U/ml) (Fig. 8B); however, IFN synthesis (about 200 U/ml) could be detected in these cells upon prolonged cultivation. We believed that this increase in IFNA2 synthesis is mediated by a ISG15 element present in our construct that is activated by constitutively produced IFNA accumulating in these cells (46). IFNA2 synthesis was, however, greatly enhanced in infected cells with the highest levels at 10 days postinfection when HIV-1 replication in both Jurkat and JISG3-A cells reached the maximal levels; IFNA2 synthesis stayed elevated in infected cells throughout the whole experiment. The levels of IFNA produced in the JISG3-A clone were affected by the multiplicity of HIV-1 infection (Table 3); however, the increase was not linear. A 20-fold increase in the multiplicity of HIV-1 infection increased the levels of IFN in the medium by only about 3-fold. These data indicate that IFNA2 synthesis can be transactivated in HIV-1-infected cells even when the levels of replicative virus are very low. The inhibition of HIV-1 replication in JISG3-A cells was not affected by an increase in virus multiplicity in the range of MOI

TABLE 2. Constitutive synthesis of IFNA2 in transfected U937 cells

Promoter	Construct ^a	No. of clones producing the following level (U/ml) of IFN/total no. of clones:			
		0–5	5–50	50–100	100–500
5' (–83) HIV-1 LTR	I	12/34	12/34	2/34	7/34
3' LTR (–83) HIV-1	II	15/40	9/40	9/40	2/40
5' ISG15 HIV-1 LTR	III	7/17	6/17	3/17	1/17
3' ISG15 HIV-1 LTR	IV	15/40	13/40	7/40	5/40

^a Constructs shown in Fig. 4.

FIG. 8. Inhibition of HIV-1 replication in Jurkat cells transduced with Tat-inducible IFNA2 gene. Jurkat cells and Jurkat clone (JISG3-A) containing integrated recombinant plasmid (vector IV in Fig. 4) were infected with HIV-1 (pLN4-3 variant) at a MOI of 0.01. (A) Medium from infected cells was collected every second day and analyzed for the presence of HIV-1 by the RT assay as described in Materials and Methods. (B) The levels of biological active IFNA2 protein were analyzed in both infected and uninfected JISG3-A cells as described in Materials and Methods.

0.025 to 0.05. Replication of HIV-1 in Jurkat cells transfected with (–83) LTR-IFNA2 (JLTR5) was not tested, since in these cells IFN synthesis was not significantly transactivated by Tat (data not shown).

Replication of HIV-1 was further examined in two clones of U937 cells, ULTR5-14 and UISG3-8, containing integrated (–83) HIV-1 LTR-IFNA2 (construct I in Fig. 4) and ISG15-HIV-1 LTR-IFNA2 (construct IV in Fig. 4), respectively. In both of these clones, synthesis of IFNA2 was greatly enhanced upon transfection with Tat-expressing plasmid (data not shown). As shown in Fig. 9A, U937 cells were permissive to HIV-1 infection, with maximal virus yield obtained about 17 days postinfection; at later times, virus production decreased because of the cytopathic effect of HIV-1, and by 21 days

postinfection, more than 70% of cells were dead. In contrast, HIV-1 replication was significantly suppressed in the ULTR5-14 clone, with only minimal virus replication at 21 days postinfection and no detectable cytopathic effect; the experiment has been carried for 40 days without detection of any cytopathic effect and significant escape of HIV-1 from the IFNA2-mediated inhibition (data not shown). The second clone tested, UISG3-8, was virtually nonpermissive to HIV-1 infection. To determine whether there is a direct correlation between the levels of synthesized IFNA2 in the infected cells and inhibition of HIV-1 replication, we measured IFN synthesis in these two clones before and after HIV-1 infection. ULTR5-14 clone produced relatively high levels of IFN constitutively (700 to 1,000 U/ml), while in UISG3-8 cells constitutive IFN synthesis was very low (25 to 100 U/ml) (Fig. 9C). The small fluctuation in IFNA2 levels seen during the cultivation of these cells represents the variability in a biological assay. A marked increase in IFNA2 production was observed upon HIV-1 infection in ULTR5-14 clone; the maximal enhancement (fivefold) of IFN synthesis was seen at 17 days postinfection, which coincided with the maximal virus production. Although the enhancement in IFNA2 synthesis was also seen in UISG3-8 cells, the relative levels were lower than in ULTR5-14 cells, and maximal enhancement (fourfold) was observed at 14 days postinfection. Thus, the difference in the degree of inhibition of HIV-1 replication between these two clones is not directly related to the levels of IFNA2 produced in these cells, and additional factors may have played a role in the resistance of the UISG3-8 clone. Interestingly, IFNA2 synthesis in the HIV-1-infected cells shows periodicity that may be directly related to the levels of replicating virus and Tat protein in these cells.

Since we could detect low levels of HIV-1 replication at later times (21 days) postinfection in ULTR5-14 cells, we were interested to determine whether this virus represents an IFNA-resistant variant. We, therefore, compared the ability of the HIV-1 isolate from the ULTR5-14 clone to replicate in U937 cells and the ULTR5-14 clone. The results in Fig. 10 show that while this HIV-1 isolate replicated effectively in U937 cells, its replication was limited in the ULTR-14 clone, suggesting that under conditions tested, this virus is still sensitive to the IFNA effect.

DISCUSSION

In a previous study (4), we have demonstrated that Tat and HIV-1 infection induced synthesis of IFN encoded by a transduced LTR-IFNA2 hybrid gene in human T cells (4) and that T cells containing the transactivable IFNA2 gene were nonpermissive to HIV-1 replication. However, HIV-1 LTR can be activated not only by Tat but also by stimuli that increase binding of the NF- κ B heterodimer to the enhancer elements of HIV-1 LTR; this mechanism was shown to play a major role in TNF- α -mediated activation of the latent HIV-1 provirus and its Tat-defective variant (43, 60). Since synthesis of TNF- α can be detected in AIDS patients, expression by the transduced HIV-1 LTR-IFNA gene *in vivo* would not be limited to HIV-1-infected cells. It was shown that the κ B motifs were not involved in Tat-mediated activation of HIV-1 LTR (36, 56); therefore, we reasoned that deletion of these two elements should minimize the induction of HIV-1 LTR by cytokines or by heterologous viral infection, both of which are mediated by NF- κ B (43, 60, 61), without affecting Tat transactivation. Surprisingly, we found that in cells permissive to HIV-1 infection, HIV-1 LTR in which the κ B motifs were deleted was not efficiently transactivated by Tat (Fig. 1A). Since in most

TABLE 3. Effect of multiplicity of HIV-1 infection on IFNA production in JISG3-A cells^a

HIV-1 MOI	IFN level (U/ml) at the following day postinfection:		
	6	8	11
0.0025	45	65	ND ^b
0.01	55	150	1,800
0.04	65	175	2,080

^a JISG3-A cells were infected with HIV-1 at the indicated MOIs, and IFNA levels in the culture medium were analyzed at 6, 8, and 11 days postinfection.

^b ND, not determined.

FIG. 9. Inhibition of HIV-1 replication in U937 cells containing the Tat-transactivable IFNA2 gene. U937 cells containing integrated (~83) HIV-1 LTR-IFNA2 plasmid (vector I in Fig. 4) and ISG15-HIV-1 LTR-IFNA2 plasmid (vector IV in Fig. 4) as well as the parental U937 cell line were infected with HIV-1 (pLN4-3) at a MOI of 0.01, and the levels of the HIV-1 virions (A) and biological active IFNA protein (B) in the medium of infected cells were determined at different times postinfection. The levels of IFNA2 in the medium of uninfected cells (C) were analyzed for comparison.

FIG. 10. HIV-1 replicating in ULTR5-14 cells is not an IFNA-insensitive variant. HIV-1 isolate from ULTR5-14 cells at 21 days postinfection (MOI of 0.01) was used to infect U937 and ULTR5-14 cells, and viral replication of this variant was analyzed by measuring the levels of RT in the medium of infected cells.

cells classical NF- κ B is present in an inactive form, it is unlikely that this factor alone plays an important role in Tat-mediated induction of HIV-1 LTR, unless activated during the course of Tat synthesis. However, recent studies have shown that the Tat protein is unable to enhance expression of the *p105* gene, which encodes the p105 precursor of the p50 subunit of NF- κ B in U937 cells (39). Thus, we can only speculate that some other cellular factor(s) binds to the κ B motifs of LTR in T cells and monocytes and cooperates with Tat to enhance transactivation.

Since deletion of the κ B motifs resulted in inefficient transactivation of LTR by the Tat protein, we examined the feasibility of increasing IFNA synthesis through enhancer activity and/or a possible feedback augmentation by the IFN-induced cellular factor ISGF-3 (30, 51) and inserted a DNA fragment from the ISG15 promoter region, containing an ISRE, upstream of HIV-1 LTR with deleted κ B motifs. Although it had been shown that the ISRE of the ISG15 promoter region is sufficient for transcriptional induction by IFNA (31), in T cells and monocytes, the natural ISG15 promoter was induced more efficiently by IFNA2 than the three copies of ISREs, suggesting that additional *cis*-acting sequences in the ISG15 promoter may contribute to its response to IFNA in these cells. The presence of ISG15 in ISG15 (–83) HIV-1 LTR significantly increased Tat activation, suggesting that the constitutively present, trans-acting factor(s) (possibly ISGF-2 [31]) bound to the ISRE enhancer(s) can cooperate with Tat. In T cells, this cooperation seems to be less efficient than that between NF- κ B and Tat, which is more than additive; activation of wild-type HIV-1 LTR by Tat was 10 times higher than that of ISG15 (–83) HIV-1 LTR (Fig. 3). In contrast, in U937 cells, Tat-mediated transactivation of ISG15 (–83) HIV-1 LTR was more efficient than that of HIV-1 LTR (Table 1). This difference in the Tat-mediated effect between monocytes and T cells may be due to the difference in constitutive levels of the *trans*-acting factor(s) interacting with the ISG15 enhancer(s). In both cell types, activation of the ISG15 (–83) HIV-1 LTR by Tat was not significantly altered by the addition of exogenous IFN, suggesting a lack of synergistic interaction between the IFNA-induced ISGF-3 complex and Tat.

One important feature of this chimeric HIV-1 LTR is the elimination of its response to the NF- κ B inducer(s), and limitation of its expression to Tat-expressing cells. Whereas the inducibility of TNF- α was abolished completely, the response

to PMA was merely decreased, since PMA activates HIV-1 LTR not only through NF- κ B but through other cellular factors as well (50). Since PMA is not a physiological inducer, it should not play a major role in the activation of this chimeric promoter *in vivo*. However, additional testing of this plasmid is needed to evaluate its response to extracellular stimuli *in vivo*.

The feasibility of using a chimeric HIV-1 LTR to direct IFN synthesis from a transduced IFN gene in HIV-1-infected cells was shown in both T cells (Jurkat) and promonocytic (U937) cells. Thus, in transiently transfected cells, expression of this modified HIV-1 LTR in the absence of Tat was either undetectable (Jurkat cells) or very low (U937). In contrast, low levels of constitutive IFNA2 synthesis (about 50 U) were observed in pools of monocytes and T cells containing integrated (–83) HIV-1 LTR or ISG15-HIV-1 LTR-IFNA2 plasmids (data not shown). However, when individual clones were analyzed, a majority of transduced clones produced low levels of IFNA constitutively (5 to 50 U/ml), while a small percentage of clones synthesized more than 100 U of IFNA2 per ml. We do not know presently whether a high level of expression of the transduced IFNA2 gene in these clones is a consequence of the presence of ISRE in the hybrid plasmid or reflects a multiple integration or position effect leading to an enhanced transcriptional activity. Our data, however, indicate that the (–83) HIV-1 LTR is not completely transcriptionally inactive in the absence of Tat protein; the high specific activity of IFNA (about 2.4×10^8 U/mg) provides a detection sensitivity not available in a conventional assay (measuring the levels of RNA transcripts).

While replication of HIV-1 was significantly suppressed in transduced cells, it was sufficient to stimulate IFNA synthesis in these cells. These data indicate that only very low levels of Tat protein are needed for transactivation of a transduced IFNA gene. Both the inhibition of HIV-1 replication and synthesis of IFNA persisted in the infected cells up to a month postinfection. Synthesized IFNA protected the infected cultures from the HIV-1-induced cytopathic changes, while it did not alter the growth rate of transduced cells (data not shown). The constitutive IFNA synthesis also did not affect the mitogenic response of these cells, as shown by their abilities to express IL-2 and IL-2R genes upon PHA and PMA stimulation.

Our study showed, however, that should the transfer of IFNA gene be used in clinical studies, the packaging of the recombinant retrovirus vector into the amphotropic virus pseudotype has to be optimized. The yields of recombinant virus produced by a majority of the selected clones were low and somewhat indirectly related to the levels of IFNA produced in transfected clones; the highest virus yield was obtained from cell clones that produced very low levels of IFNA. We have previously shown (40) that assembly of murine retrovirus is inhibited in IFN-treated cells, but we expected that the low levels of constitutively produced human IFNA in the murine packaging line would not have a major effect on the assembly of the recombinant virus. Additional studies will have to determine whether a high yield of recombinant virus could be obtained in the presence of anti-human IFNA antibodies or whether other viral vectors may provide an alternative, more effective strategy for delivery of IFNA genes.

The IFNA-mediated inhibition of HIV-1 replication shows several unique features compared with the other HIV-1 inhibitors used in the gene therapy approach. First, the long-lasting suppression of HIV-1 replication in cells that synthesize IFNA2 in an autocrine fashion suggests that the formation of the IFN-resistant HIV-1 phenotype is not acquired as easily as resistance to nucleoside inhibitors or ribozymes is (37, 52). We

have previously shown that in a single-step cycle infection, IFNA inhibits HIV-1 replication on a preintegrational step at the level of provirus formation (55). Similarly, in cells transduced with the transactivable IFN gene, there was marked inhibition of provirus integration and expression (data not shown). The inability of HIV-1 to escape IFNA-mediated inhibition easily suggests that the formation of the IFN-resistant HIV-1 phenotype may involve larger changes than point mutations found in zidovudine- and ribozyme-resistant HIV-1 variants (37, 52). Second, in contrast to ribozymes or dominant negative mutants that have been used for intracellular "immunization," transduction of the IFNA2 gene is associated with the amplification effect mediated by a released IFNA. The ability of IFNA to inhibit HIV-1 replication by both the autocrine and paracrine mechanisms would be of a great advantage for its use *in vivo*, where transduction of a protective gene into every cell is not easily attainable.

In summary, we have shown that the modified ISG15-HIV-1 LTR promoter is able to direct high levels of IFNA2 synthesis in human T cells and monocytes infected with HIV-1. Unlike endogenous IFNA, expression of the transduced ISG15-HIV-1 LTR-IFNA gene is induced by HIV-1 infection, and its induction is further enhanced by IFNA; in contrast to the natural HIV-1 LTR, the chimeric HIV-1 LTR is not stimulated by NF- κ B-specific activators such as TNF- α . It will be of interest to examine whether primary PBMC or macrophages containing the transduced ISG15-LTR-IFNA2 hybrid gene are resistant to HIV-1 infection and retain their immune functions. These experiments are currently under way in our laboratory.

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