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Inhibition of human immunodeficiency virus (HIV) replication by HIV-trans-activated α_2-interferon

(antiviral state/antiviral modality/site-directed synthesis)

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ABSTRACT **We have prepared stable cell lines, derived from Vero cells and A3.01 cells, that express a hybrid human α_2-interferon gene under control of the human immunodeficiency virus (HIV) long terminal repeat. These cells constitutively produced low levels (50–150 units/ml) of α_2-interferon. However, high levels of interferon (10^3 units/ml) could be induced upon trans-activation by the product of the *tat* gene (pIIIextatIII), and *de novo* infection by HIV resulted in a moderate increase (400 units/ml) in α_2-interferon synthesis. In contrast to the fully permissive HIV replication, in transfected Vero cells or infected A3.01 cells, the transcription and replication of HIV in Vero or A3.01 cells containing the HIV long terminal repeat–α_2-interferon hybrid gene (VN89 and A3N89 cells, respectively) was completely inhibited. These data suggest that virus-trans-activated α_2-interferon synthesis can be used as a selective inhibitor of HIV replication.**

It has been well established that the human immunodeficiency virus (HIV) is the etiologic agent of acquired immunodeficiency syndrome (AIDS) (1–5). Although substantial progress has been made on the molecular characterization of this virus, progress toward therapeutic or prophylactic treatment remains uncertain. The unique feature of HIV infection that permits this virus to remain quiescent for a long period of time (6–10) presents obstacles to therapeutic or prophylactic regimens.

In this study, we examined the feasibility of the interferon system to act as an inhibitor of HIV replication. We believe that interferon has the potential to inhibit the replication of HIV and other unrelated viruses that have been demonstrated to activate the replication of latent HIV (10–13). Interferon can inhibit acute murine leukemia virus (MuLV) infection, iododeoxyuridine-induced endogenous MuLV activation, and chronic MuLV infection (14, 15). In contrast to the interferon effect on most of the lytic viruses, interferon did not inhibit MuLV-specific protein synthesis or prevent the establishment of infection but altered the fidelity of virus assembly with resumed virus production upon interferon removal (14). In interferon-treated cells, noninfectious virus particles were formed that lacked the viral glycoprotein gp71 (16). HIV replication *in vitro* was also shown to be sensitive to interferon; however, this inhibition is moderate and reversible upon interferon removal (17–20). To achieve prolonged high concentrations of interferon at the site of infection, we employed a retroviral vector-mediated "gene therapy" approach and introduced into cells the human α_2-interferon gene, as the antiviral modality, under the control of the HIV long terminal repeat (LTR). Furthermore, we reasoned that the interferon encoded by this hybrid gene should be synthesized effectively only in HIV-infected cells but not in the uninfected cells. This would provide selectivity not obtainable with exogenous interferon. The results of this study demonstrate that cell lines containing the integrated HIV LTR–α_2-interferon hybrid gene constitutively produced low levels of α_2-interferon and that this gene could be trans-activated by the *tat* gene product or by HIV infection. Furthermore, cells containing the HIV LTR–α_2-interferon hybrid gene were resistant to HIV replication, whereas the parental cell lines were fully permissive.

MATERIALS AND METHODS

Cells and Viruses. Vero cells (African green monkey kidney cell line) were grown as described (10–12). A3.01 cells ($CD4^+$ CEM T-cell line) were maintained in OPTI-MEM (GIBCO) medium supplemented with 2.0% (vol/vol) fetal bovine serum. Stock cultures of HIV-1 were generated by electroporation of 10 μg of an infectious HIV DNA clone (pHXBC2) into A3.01 cells. The virus collected in the medium 7 days after electroporation was fully infectious; its concentration, determined by HIV-1 p24 antigen capture assay (Abbott), was higher than 2000 pg/ml. *De novo* infection of A3.01 cells was carried out by inoculating 1 ml of HIV stock into 20 ml of cells (10^6 cells per ml) under these conditions, maximal levels of HIV were produced in the cultures 8–10 days after inoculation.

Plasmid DNA and Construction of Retroviral Expression Vectors. The HIV LTR sequences were obtained as the *Bam*HI–*Hin*dIII fragment of pU3RIIICAT (HIV LTR–CAT) plasmid DNA, where CAT is chloramphenicol acetyltransferase (21). The human α_2-interferon sequence was obtained from the pCR122 plasmid (22, 28) containing the genomic clone of the human α_2-interferon gene by digestion with *Sau* I at a site 20 nucleotides downstream of the cap site. After addition of *Hin*dIII linker DNA, further digestion with *Hin*dIII and *Eco*RI (at a site 65 nucleotides downstream of the α_2-interferon gene polyadenylylation site), generated a 1000-base-pair (bp) fragment that was cloned between the *Hin*dIII and *Eco*RI sites of pU3RIIICAT to yield pHIVα2. The HIV α_2-interferon fusion gene was inserted into modified retroviral vector pLJ (23) by digestion of pHIVα2 with *Xho* I and *Eco*RI, ligation of *Bam*HI linkers, and subsequent *Bam*HI digestion. The isolated 1750-bp fragment was cloned into the unique *Bam*HI site of modified pLJ retroviral vector DNA. The modified pLJ vector was derived from the pLJ vector of R. Mulligan and coworkers (23) by deletion of the enhancer sequences in the 3′ LTR, as described (24), and contains the neomycin-resistance gene (neo) under the control of the simian virus 40 early promoter region (Fig. 1).

Abbreviations: LTR, long terminal repeat; HIV, human immunodeficiency virus; VN88/89, neomycin-resistant Vero cell line expressing α_2-interferon under the direction of HIV LTR; A3N88/89, neomycin-resistant A3.01 cell line expressing α_2-interferon under the direction of HIV LTR; MuLV, murine leukemia virus; CAT, chloramphenicol acetyltransferase.

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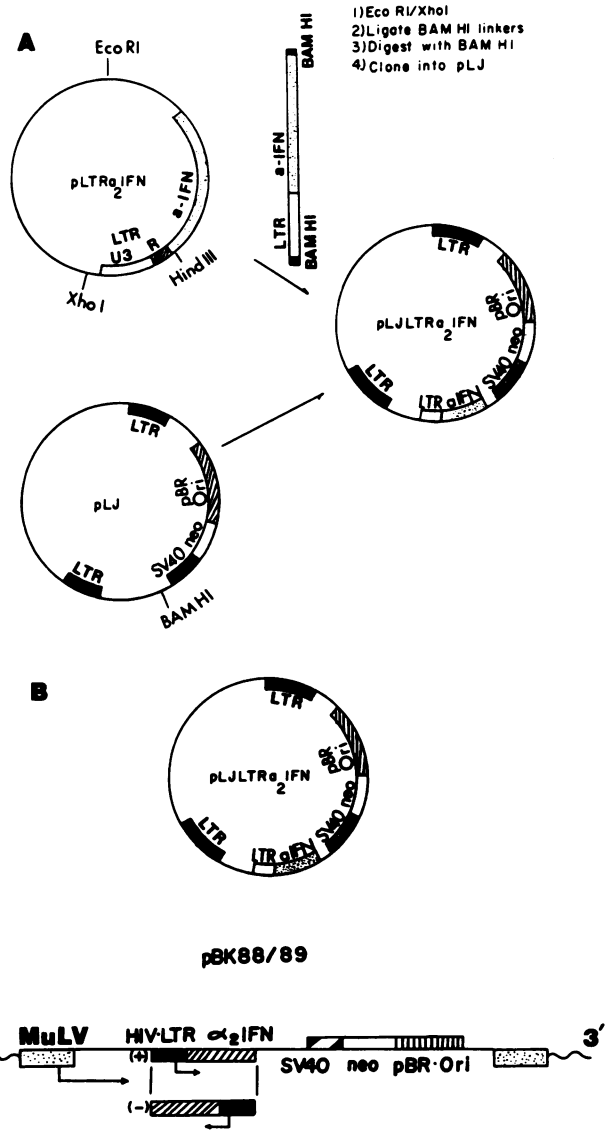

FIG. 1. Construction of retroviral expression vectors containing human α_2-interferon (α-IFN or α_2-IFN) under control of the HIV LTR. (*A*) The 1-kb fragment containing the α_2-interferon gene was inserted behind HIV LTR and the LTR–α_2 fusion gene was cloned into the unique *Bam*HI site of modified pLJ (3′ en$^-$) vector DNA (23). (*B*) The construction yielded two orientations of the insert denoted (+) for pBK88 and (−) for pBK89 in the forward and inverted orientations, respectively. The arrows indicate the possible directions of transcript initiation. neo, Neomycin-resistance gene; Ori, origin of replication; SV40, simian virus 40.

Construction of Permanent Cell Lines. DNA was transfected into Vero cells or ΨAm cells (25) as described (10–12) or electroporated into A3.01 cells (400 V and 800 millifarads for 10 msec). Permanent Vero or A3.01 cell lines were established by using the retroviral shuttle vector technique (24, 25). Vero cells containing the integrated HIV–α_2 hybrid genes were obtained by infection with recombinant virus followed by selection in medium containing G418 antibiotic (1 mg/ml). Infected permanent lymphoid (A3.01) cell lines were selected by their ability to grow in semi-solid suspensions (0.1% agar, Difco) containing G418 (1 mg/ml).

RESULTS

Expression of α_2-Interferon Under Control of the HIV LTR. We have constructed a hybrid gene in which expression of the human α_2-interferon gene was directed by the HIV 3′ LTR and could be activated in trans by the product of the *tat* gene. To facilitate the transfer of this hybrid gene into various types of cells, the HIV LTR–α_2-interferon hybrid gene was inserted into the pLJ vector either in the forward (+) or inverted (−) orientation with respect to the MuLV LTR (Fig. 1). To ensure that the transcription of the α_2-interferon gene initiated from HIV LTR and not in the MuLV LTR of the retroviral vector, the enhancer region of the 3′ LTR of the pLJ vector was deleted (J. Engelhardt and P.M.P., unpublished results). The retroviral vector containing the HIV LTR–α_2-interferon hybrid was transmitted as a defective amphotropic retrovirus into Vero cells (simian) and A3.01 (human) T cells. Vero cells contain (26) a homozygous deletion of type I (α and β) interferon genes, and thus any interferon effect observed in these cells must be encoded by the introduced α_2-interferon hybrid gene. A3.01 cells expressing CD4 surface antigen can be readily infected by HIV; in these cells, virus induces cytopathic effects and cell death (27).

Permanent Vero and A3.01 cell lines containing the HIV LTR–α_2-interferon sequences were further studied (Fig. 2). Expression of the HIV LTR–α_2-interferon gene before and after trans-activation by the product of pIIIextatIII plasmid was analyzed both on the RNA level by Northern blot hybridization of total cellular RNA and on the protein level by bioassay of α_2-interferon. Cell lines, VN88 and A3N88, (Vero and A3.01 cells, respectively) that contained the forward (+) orientation of the HIV LTR–α_2-interferon hybrid gene within the retroviral vector constitutively expressed a 2.4-kilobase (kb) α_2-interferon transcript. This hybrid gene was not trans-activated by the product of the *tat* gene after transfection with pIIIextatIII plasmid. The 2.4-kb mRNA was also detected by hybridization with an MuLV-specific probe (data not shown), indicating that these transcripts were initiated from the murine LTR (Fig. 2*A*). Both cell lines constitutively produced α_2-interferon at 50–150 units/ml before and after transfection with pIIIextatIII. The VN89 and A3N89 cell lines, which contained the inverted (−) orientation of the hybrid gene, expressed a 1.1-kb α_2-interferon mRNA (Fig. 2*B*). This mRNA was correctly initiated from the HIV LTR as shown by S1 nuclease analysis (Fig. 2*C*). Furthermore, the relative levels of α_2-interferon mRNA were significantly enhanced upon *tat*-mediated trans-activation. The enhancement of α_2-interferon gene expression after trans-activation could be demonstrated also on the protein level. Whereas the VN89 and A3N89 cells constitutively produced α_2-interferon at 50–150 units/ml after transfection with pIIIextatIII the levels of α_2-interferon synthesized were about 10^3 units/ml (Fig. 2*B*). These results indicate that the response of the HIV LTR–α_2-interferon hybrid to trans-activation depends on its orientation in the retroviral vector.

Effect of HIV-Trans-activated α_2-Interferon Synthesis on HIV Replication. To test the efficacy of HIV trans-activated α_2-interferon as an inhibitor of HIV replication, the VN88 and VN89 cell lines were transfected with full-size HIV infectious clone DNA (pHXBC2) or the A3N88 and A3N89 cell lines were directly infected with HIV. Replication of HIV in cells containing the human α_2-interferon hybrid gene and in the parental cell lines was monitored on the RNA level by Northern blot hybridization analysis of viral transcripts and by the release of virus particles into the medium. Vero cells were fully permissive to HIV replication after transfection of infectious clone DNA (Table 1), and virus production could be detected in the medium both by antigen capture and reverse transcriptase assays. When the infectious proviral DNA was transfected into VN89 cells, no virus particles were detected in the medium (Table 1). Also, when the transfected VN89 cells were cocultivated with A3.01 cells, no virus progeny or cytopathic effect could be detected in A3.01 cells after 5–10 days of coculture (Table 1). The replication of HIV

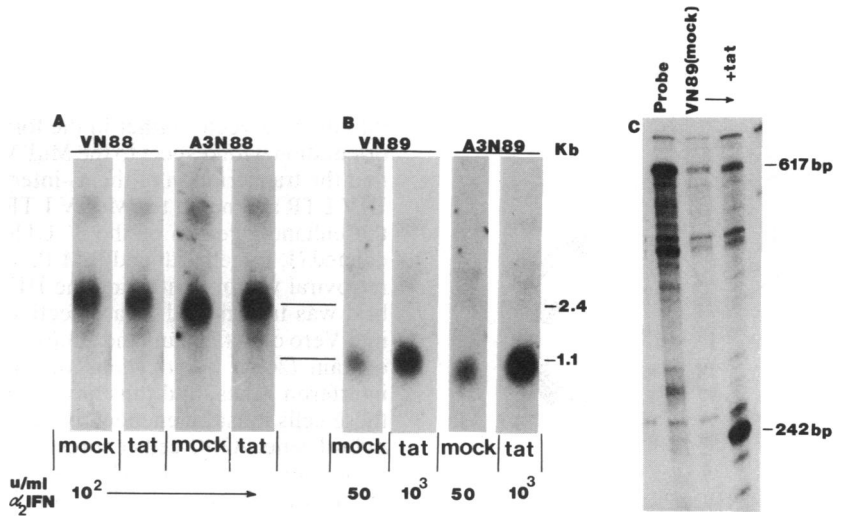

FIG. 2. Analysis of relative levels of α_2-interferon mRNA in Vero or A3.01 cells containing integrated pBK88 or pBK89 DNA before and after transfection with pIIIextatIII. Total RNA was isolated from VN88 or A3N88 cells containing the pBK88 plasmid (*A*) and from VN89 or A3N89 cells containing the pBK89 plasmid DNA (*B*) before (lanes mock) and 24 hr after transfection with pIIIextatIII (lanes tat) as described (11). RNA (10 μg) was analyzed by Northern blot hybridization with a human α_2-interferon riboprobe (28). The levels of secreted human α_2-interferon, assayed by the cytopathic method (36), are shown below each panel and expressed as units/ml. S1 nuclease analysis of the correctly initiated HIV LTR–α_2-interferon transcript in VN89 cells transfected with pIIIextatIII (lane +tat) or mock-transfected with pBR322 DNA is shown in *C*. The positions of the full-length probe (617 bp) and the correctly initiated HIV LTR–α_2-interferon transcript (242 bp) are indicated in *C*.

in VN88 cells was also markedly reduced yielding only 35% of control cultures. Spreading of infection and cytopathic effects were also significantly reduced when A3.01 cells were cocultured with VN88 as compared to HIV-producing Vero cells cocultivated with A3.01 cells (Table 1).

To verify that the inhibition of HIV production was due to the α_2-interferon and not simply a consequence of the presence of HIV LTR elements competing for transcriptional factors, we examined the replication of infectious proviral DNA in permanent cell lines containing the HIV LTR–CAT fusion gene (VNCAT) (10–12) or in cells containing the pLJ murine retroviral vector lacking an interferon insert (VN-pLJ). Results shown in Table 1 demonstrate that both cell lines are permissive to HIV replication, thereby eliminating this possibility.

To evaluate the effect of the trans-activated human α_2-interferon in human T cells, the A3.01, A3N88, and A3N89 cell lines were inoculated *de novo* with HIV and virus replication (assayed by the presence of p24 antigen in the medium) was measured over a 14-day period (Fig. 3). In A3.01 cells, virus production reached maximum 6–7 days after infection. The A3N89 cell line, in which the synthesis of α_2-interferon could be trans-activated by *tat*, was not permissive for HIV infection and showed no evidence of HIV replication or HIV-induced cell fusion over a period of 14 days after infection. To ensure that the inhibition of HIV replication in A3N89 cells was related to the synthesis of α_2-interferon and was not simply an artifact resulting from the cloning of a Leu3a$^-$ (CD4 defective) population (27), we compared the expression of cell-surface CD4 antigen on A3.01 and A3N89 cells by fluorescence-activated cell sorting (FACS; Fig. 3 *Inset*). The results showed that after incubation with a fluorescein isothiocyanate-labeled Leu3a monoclonal antibody, more than 99% of the A3N89 clonal population displayed Leu3a-dependent cell surface fluorescence, indicating that the levels of CD4 surface antigen in A3.01 cells and A3N89 cells are about the same.

Table 1. Expression of HIV-1 p24 antigen and reverse transcriptase activity by transfected Vero or infected A3.01 cell lines

Cell line	HIV p24 capture, pg/ml	Reverse transcriptase, cpm/ml
Vero	>1000	$>10^6$
VN89	<5.0	500
VNCAT	826	8×10^5
VNpLJ	>1000	$>10^6$
Vero/A3.01	1000	$>10^6$
VN88/A3.01	280	10^4
VN89/A3.01	<5.0	500
A3.01	>1000	ND
A3N89.9	<5.0	ND
A3N89.7	<5.0	ND
A3N89.12	<5.0	ND

Reverse transcriptase activity was determined as described (29). ND, not determined.

To determine the level at which the interferon-induced block of HIV replication occurs, HIV transcripts were analyzed in the infected cells. Northern blot analysis (Fig. 4*A*) of total RNA isolated from Vero or VN89 cells 24 hr after transfection with HIV proviral DNA (pHXBC2) demonstrated that, in transfected Vero cells, both genomic and spliced HIV mRNAs could be detected. When transfected VN89 cells were analyzed, the levels of both genomic and spliced mRNAs were very low and faint bands could be detected only upon prolonged exposure. Although accumulation of HIV transcripts was inhibited, the relative levels of cellular actin mRNA were unchanged (Fig. 4*B*), indicating the presence of a selective antiviral mechanism. Similarly, when A3N89 clones were infected with HIV, no apparent accumulation of HIV transcripts was evident even after 7–14 days in culture (Fig. 4*C*). Low levels of hybridization seen at approximately 5 kb were attributed to nonspecific binding of the riboprobe to rRNA. In parental A3.01 cells, however, the genomic and spliced viral mRNAs could be easily detected. In contrast to A3.01 cells where the HIV infection causes a marked cytopathic effect, continuous culture of HIV-infected A3N89 cells showed no signs of cell death (greater than 95% viability) over a 14-day period and these cultures never produced virus progeny (Fig. 3). Although interferons

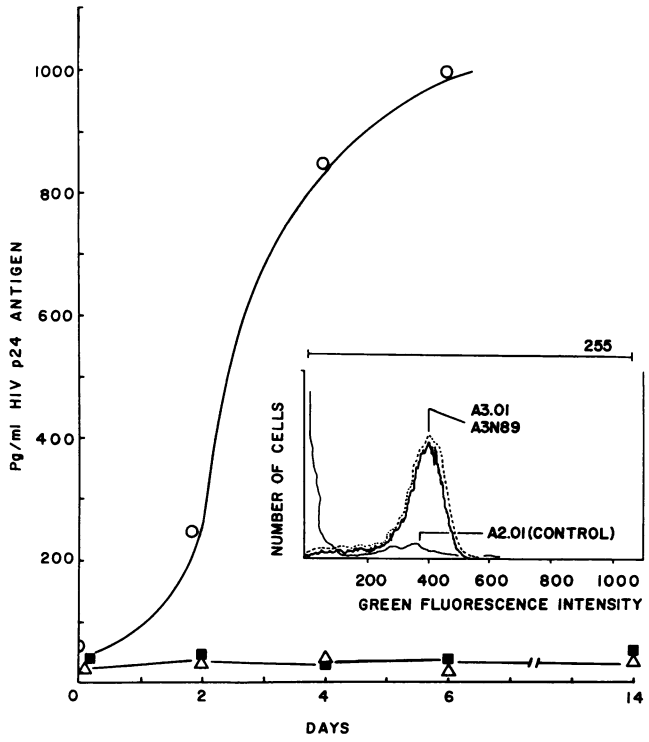

FIG. 3. Time course of HIV-1 p24 production by infected A3.01 and A3N89 cells: Expression of cell-surface CD4 receptors on A3.01 and A3N89 cells. A3.01 control cultures and A3N89 cells were infected with HIV. The virus present in the cell-free supernatants was determined by the immunocapture method (Abbott) (30). Open circles, medium harvested from infected A3.01 cells; closed squares, from uninfected A3.01 cultures; open triangles, A3N89 cells. (*Inset*) The presence of cell-surface CD4 receptor on A3.01 and A3N89 cells was analyzed by staining with fluorescein isothiocyanate-labeled Leu3A monoclonal antibody (4°C for 30 min) and measured on a Coulter–Epics model −742 fluorescence-activated cell sorter (FACS). The relative fluorescence intensity versus cell number are shown, and the upper horizontal bar indicates channels analyzed. The control represents staining of leu3A$^-$ A2.01 cells. Solid lines, A3.01 cells; dashed line, A3N89 cells.

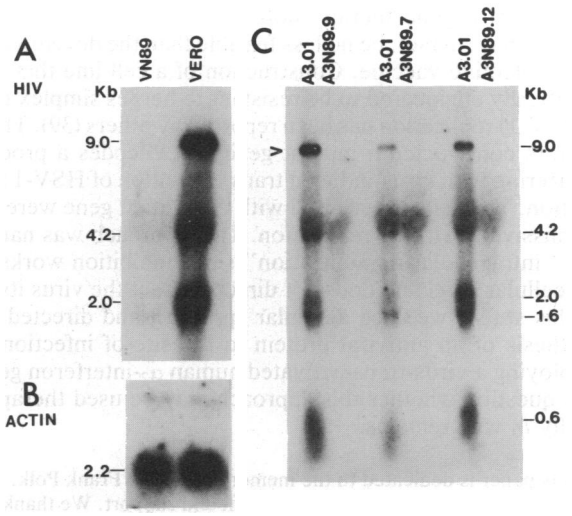

FIG. 4. Analysis of the relative levels of HIV transcripts in transfected Vero and VN89 cells or infected A3.01 and A3N89 cells. (*A*) RNA was isolated from Vero or VN89 cells (10^7 cells) transfected with 10 μg of pHBCX2 DNA 36 hr after transfection. RNA (10 μg) was analyzed by Northern blot hybridization with an HIV riboprobe (pJM105). (*B*) Identical membrane described in *A* was hybridized with a ^{32}P-labeled actin DNA probe. (*C*) RNA (10 μg) isolated from A3.01 parental cells and three of the A3N89 clones 7 days after the infection was analyzed for the presence of viral transcripts as described in *A*.

are well characterized antimitotic factors (31), there were no growth effects on these cell lines (data not shown).

DISCUSSION

The experiments presented in this report show that α_2-interferon-mediated inhibition of HIV production is more effective when generated *in situ* than when interferon is added exogenously.

We have established a system that allows the expression of interferon in HIV-infected cells. The transfer of genes by use of defective retroviruses has been explored by others as a potential vehicle for gene therapy (23). With few exceptions (24), retroviruses have not been used as vectors for the transfer of genes carrying their own regulatory promoters. Our results show that, when the HIV LTR–α_2-interferon hybrid gene was inserted in the forward (+) orientation, transcription was initiated in the vector MuLV LTR. This construct was unable to respond to transcriptional trans-activation by the *tat* gene product. Only when transcription of the α_2-interferon gene was initiated in the HIV LTR [the hybrid gene inserted in the inverted (−) orientation] was its expression enhanced by trans-activation.

The trans-activation of α_2-interferon expression and synthesis by HIV resembles the induction of native interferon by other unrelated viruses, a process that does not occur efficiently in cells during HIV infection (unpublished data). Transfection of VN89 or A3N89 cells with pIIIextatIII caused the synthesis and secretion of α_2-interferon of up to 10^3 units/ml; however, HIV infection resulted in the production of only about 400 units/ml. Therefore, synthesis of α_2-interferon was directly proportional to the viral *tat* gene product, making the system self-regulating.

The antiviral activity conferred on Vero (VN89) and A3.01 (A3N89) cells was attributed to the action of α_2-interferon since our studies revealed that HIV replicated well in other cell lines containing the murine retroviral vector lacking the HIV LTR–α_2-interferon insert (VNpLJ) or in cells containing stably transfected HIV LTR–CAT (VNCAT) (10–12). The presence of *tat* also dramatically elevated the levels of 2′–5′ oligoadenylate synthetase and interferon stimulated gene 15 (ISG-15) mRNAs in A3N89 cells (data not shown). Transcription of both of these genes was shown to be stimulated by interferon in human cells (31–35). In addition, all 12 clones tested expressed cell surface CD4 antigen at levels equal to or greater than parental A3.01 cells. This eliminated the possibility of cloning a receptor-defective cell line. Although the action of interferons on retrovirus replication has been localized to the process of assembly and maturation (14–16), the mechanism of HIV inhibition in cells expressing the trans-activated α_2-interferon appeared to be at the level of viral transcription or mRNA stability. Expression of α_2-interferon by this construct did not significantly alter growth characteristics of the cells employed in this study. The addition of the anti-α-interferon antibodies to the medium of VN89 cells that were transfected with the infectious HIV clone DNA did not reverse the inhibitory effect. The effect of interferon in the producing cell is dependent on its release from the cells and its interaction with the cellular membrane (36). It is possible, however, that interferon produced from this vector can have an additional site of action.

As the number of AIDS patients continues to rise, the development of effective treatment and prophylaxis has become urgent. Other studies have shown that conjugation of the soluble virus receptor (CD4) with a recombinant human CD4$^-$ pseudomonas exotoxin hybrid protein or rCD4-ricin A specifically killed HIV-infected cells *in vitro* (37, 38). The

concept of a gene therapy approach for treatment of this disease may, in fact, be no less feasible than the development of an effective vaccine. Construction of a cell line that was genetically engineered to be resistant to herpes simplex type 1 (HSV-1) replication has been reported by others (39). These authors constructed a mutant gene that encodes a product interfering with virus-induced trans-activation of HSV-1 replication. The cells transfected with the mutant gene were not permissive to HSV-1 replication. This approach was named (40) "intracellular immunization" since inhibition works on the cellular level and does not directly affect the virus itself. In this study, we used a similar approach and directed the synthesis of an antiviral protein to the site of infection by employing a virus-trans-activated human α_2-interferon gene. The question whether this approach may be used therapeutically *in vivo* remains.

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1. Barre-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W. & Montagnier, L. (1983) *Science* **220,** 868–871.
2. Popovic, M., Sarngadharan, M. G., Read, E. & Gallo, R. C. (1984) *Science* **224,** 497–500.
3. Gallo, R. C., Salahuddin, S. Z., Popovic, M., Shearer, G. M., Kaplan, M., Haynes, B. F., Palker, T. J., Redfield, R., Oleske, J., Safai, B., White, G., Foster, P. & Markham, P. D. (1984) *Science* **224,** 500–503.
4. Broder, S. (1984) *N. Engl. J. Med.* **318,** 243–245.
5. Sarngadharan, M. G., Popovic, M., Bruch, L., Schupbach, J. & Gallo, R. C. (1984) *Science* **224,** 506–508.
6. Curran, J. W., Morgan, W. M., Hardy, A. M., Jaffe, H. W., Darrow, W. W. & Dowdle, W. R. (1985) *Science* **229,** 1352–1357.
7. Cao, Y., Valentine, F., Hojvat, S., Allain, J.-P., Rubinstein, P., Mirabile, M., Czelusniak, S., Leuther, M., Baker, L. & Friedman-Kien, A. E. (1987) *Blood* **70,** 575–578.
8. Goudsmit, J., Lange, J. M., Paul, D. A. & Dawson, G. J. (1987) *J. Infect. Dis.* **155,** 558–560.
9. Price, R. W., Brew, B., Sidtis, J., Rosenblum, M., Scheck, A. C. & Cleary, P. (1988) *Science* **239,** 586–592.
10. Bednarik, D. P., Mosca, J. D. & Raj, N. B. K. (1987) *J. Virol.* **61,** 1253–1257.
11. Mosca, J. D., Bednarik, D. P., Raj, N. B. K., Rosen, C. A., Sodroski, J. G., Haseltine, W. A., Hayward, G. S. & Pitha, P. M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7408–7412.
12. Mosca, J. D., Bednarik, D. P., Raj, N. B. K., Rosen, C. A., Sodroski, J. G., Haseltine, W. A. & Pitha, P. M. (1987) *Nature (London)* **325,** 67–70.
13. Gendelman, H. E., Phelps, W., Feigenbaum, L., Ostrove, J. M., Adachi, A., Howley, P. M., Khoury, G., Ginsberg, H. S. & Martin, M. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9759–9763.
14. Pitha, P. M., Rowe, W. P. & Oxman, M. N. (1976) *Virology* **70,** 324–338.
15. Friedman, R. M. & Ramseur, J. M. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3542–3544.
16. Bilello, J. A., Wivel, N. A. & Pitha, P. M. (1982) *J. Virol.* **43,** 213–222.
17. Bednarik, D. P. & Pitha, P. M. (1988) in *The Biology of the Interferon System* , ed. Kawade, Y. & Kobayashi, S. (ISIR-JSIR, Japan), pp. 109–116.
18. Ho, D. D., Rota, T. R., Kaplan, J. C., Hartshorn, K. L., Andrews, C. A., Schooley, R. T. & Hirsch, M. S. (1985) *Lancet* **ii,** 602–604.
19. Yamamoto, J. K., Barre-Sinoussi, F., Bolton, V., Pedersen, N. C. & Gardner, M. B. (1986) *J. Interferon Res.* **6,** 143–152.
20. Pomerantz, R. J. & Hirsch, M. S. (1987) *Interferon* **9,** 113–127.
21. Rosen, C. A., Sodroski, J. G. & Haseltine, W. A. (1985) *Cell* **41,** 813–823.
22. Goeddel, D. V., Leung, D. W., Dull, T. J., Gross, M., Lawn, R. M., McCandliss, R., Seeburg, P. H., Ullrich, A., Yelverton, E. & Gray, P. W. (1981) *Nature (London)* **290,** 20–26.
23. Korman, A. J., Frantz, J. D., Strominger, J. L. & Mulligan, R. C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2150–2154.
24. Dzierzak, E. A., Papayannopoulu, T. & Mulligan, R. C. (1988) *Nature (London)* **331,** 35–41.
25. Miller, A. D. & Buttimore, C. (1986) *Mol. Cell. Biol.* **6,** 2895–2902.
26. Diaz, M. O., Zieman, L., Le Beau, M. M., Pitha, P., Smith, S. D., Chilcote, R. R. & Rowley, J. D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5259–5263.
27. Folks, T., Powell, D. M., Lightfoote, M. M., Benn, S., Martin, M. A. & Fauci, A. S. (1986) *Science* **231,** 600–602.
28. Raj, N. B. K., Kellum, M., Kelley, K. A., Antrobus, S. & Pitha, P. M. (1985) *J. Interferon Res.* **5,** 493–510.
29. Adachi, A., Gendelman, H. E., Koenig, S., Folks, T., Willey, R., Rabson, A. & Martin, M. A. (1986) *J. Virol.* **59,** 284–291.
30. Higgins, J. R., Pederson, N. C. & Carlson, J. R. (1986) *J. Clin. Microbiol.* **24,** 424–430.
31. Chen, L., Mory, Y., Zilberstein, A. & Revel, M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8037–8041.
32. Hovanessian, A. G. & Kerr, I. M. (1979) *Eur. J. Biochem.* **93,** 515–526.
33. Lengyel, P. (1982) *Ann. Rev. Biochem.* **51,** 251–282.
34. Benech, P., Merlin, G., Revel, M. & Chabath, J. (1985) *Nucleic Acids Res.* **13,** 1267–1281.
35. Blomstrom, D. C., Fahey, D., Kutny, R., Korant, B. D. & Knight, E. (1986) *J. Biol. Chem.* **261,** 8811–8816.
36. Vengris, V. E., Stollar, D. & Pitha, P. M. (1975) *Virology* **65,** 410–417.
37. Till, M. A., Ghetie, V., Gregory, T., Patzer, E. J., Porter, J. P., Uhr, J. W., Capon, D. J. & Vitteta, E. S. (1988) *Science* **242,** 1166–1168.
38. Chaudhary, V. K., Mizukami, T., Fuerst, T. R., Fitzgerald, D. J., Moss, B., Pastan, I. & Berger, E. A. (1988) *Nature (London)* **335,** 369–372.
39. Friedman, A. D., Trienzberg, S. J. & McKnight, S. L. (1988) *Nature (London)* **335,** 452–454.
40. Baltimore, D. (1988) *Nature (London)* **335,** 395–396.