JOURNAL OF VIROLOGY, July 1996, p. 4871–4876 Vol. 70, No. 7
0022-538X/96/$04.00+0

Inhibition of Human Immunodeficiency Virus Type 1 Replication Is Enhanced by a Combination of Transdominant Tat and Rev Proteins

CATHERINE ULICH,[1] DAVID HARRICH,[1] PILA ESTES,[2] AND RICHARD B. GAYNOR[1]*

Divisions of Molecular Virology and Hematology-Oncology, Departments of Medicine and Microbiology,[1] and Department of Pathology,[2] University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235-8594

Received 19 January 1996/Accepted 9 April 1996

Mutation of either of two critical human immunodeficiency virus type 1 (HIV-1) regulatory proteins, Tat and Rev, results in marked defects in viral replication. Thus, inhibition of the function of one or both of these proteins can significantly inhibit viral growth. In the present study, we constructed a novel transdominant Tat mutant protein and compared its efficiency in inhibiting HIV-1 replication with that of transdominant mutant Rev M10 when these proteins were stably expressed either alone or in combination in T-lymphocyte cell lines. The transdominant Tat mutant protein alone resulted in a modest inhibition of HIV replication, but it was able to enhance the ability of the M10 Rev mutant protein to inhibit HIV-1 replication. These results suggest a possible synergistic effect of these transdominant mutant proteins in inhibiting HIV-1 replication.

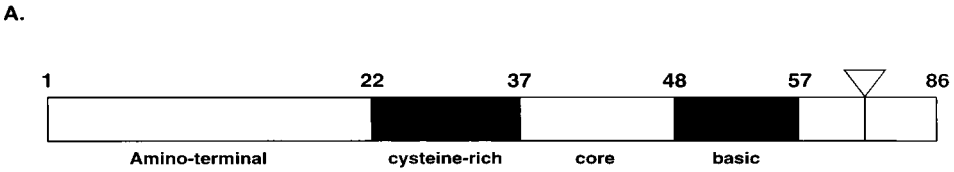
Mutant regulatory or structural proteins known as transdominant or dominant negative mutant proteins have efficacy in preventing human immunodeficiency virus type 1 (HIV-1) replication. Transdominant proteins have been described for a variety of HIV-1 proteins, including Tat (36, 41), Rev (6, 20, 23, 30, 32, 38, 49), and Gag (48). These mutant proteins are defective in their normal function but are able to inhibit the activity of their corresponding wild-type protein by either forming inactive multimers with the wild-type protein or binding to a limiting cellular factor that is critical for the function of the wild-type protein (4). Transdominant mutant proteins must be present in increased concentrations relative to those of the respective wild-type proteins to effectively inhibit the function of the wild-type protein. Two excellent targets for transdominant inhibition are the regulatory proteins Tat and Rev. Mutations which inactivate either of these proteins result in viruses that are unable to efficiently replicate (10, 14, 15, 45, 47). Thus, the development of effective inhibitors of one or both of these proteins may have utility in inhibiting HIV-1 replication.

Rev is a 116-amino-acid nuclear protein that is responsible for the temporal expression of HIV-1 structural proteins (6, 20, 22, 23, 29, 31–33). Rev binds to a specific RNA structure, the Rev response element, located within the envelope gene (33, 39, 40, 51) either to prevent the splicing of full-length viral RNAs or to increase the cytoplasmic transport of these viral RNAs. Recent observations suggest that the Rev protein can shuttle between the cytoplasm and the nucleus (34) and that a nuclear pore protein may be a critical cellular target for Rev interaction (7, 16). Rev contains an arginine-rich domain necessary for both oligomerization and nuclear localization (2, 21, 28) and an activation domain which contains critical leucine residues (2, 21, 28). Mutations in the leucine domain of Rev result in proteins that are very defective for Rev function but that have a transdominant phenotype (2, 21, 28, 33, 51). This outcome is evidenced by the ability of these proteins to bind to the Rev response element and multimerize and by their failure to interact with cellular factors involved in RNA transport. One such mutant protein known as Rev M10 inhibits wild-type Rev function, and when it is expressed in T-cell lines and peripheral blood lymphocytes, a markedly reduced HIV-1 replication results (3, 5, 28, 30, 49).

The HIV-1 and HIV-2 Tat proteins contain a number of distinct domains, including amino-terminal, cysteine-rich, core, basic, and auxiliary domains (9). The basic domain mediates Tat binding to Tat activation response element (TAR) RNA and also functions in nuclear localization (8, 46). Mutations which either delete the HIV-1 and HIV-2 Tat basic domain or alter the charge of this domain by substitution of neutral amino acids result in proteins that are very defective in activating HIV gene expression (11, 17, 43, 44). However, these mutations result in transdominant proteins which inhibit wild-type Tat activation of HIV gene expression (12, 36, 41). An earlier study demonstrated that a Tat protein that had been truncated at amino acid 53, resulting in the removal of the majority of the Tat basic domain, failed to prevent HIV-1 replication (3). However, neutral amino acid substitutions between amino acids 52 and 57 in the Tat basic domain were much more effective than the Tat truncation mutations in producing proteins that inhibit wild-type Tat activation of HIV-1 gene expression (36).

It was important to determine whether removal of further Tat basic amino acids would result in even a stronger transdominant phenotype. Two remaining lysine residues in the basic domain of the Tat transdominant mutant 52/57 protein were changed to glycine residues to construct the Tat mutant 50/57 protein (Fig. 1). To analyze whether the Tat mutant 50/57 protein was a stronger antagonist of wild-type Tat activation than the Tat 52/57 protein, transient transfections were performed in HeLa cells. An HIV-1 long terminal repeat (LTR) chloramphenicol acetyltransferase (CAT) reporter was cotransfected with Rous sarcoma virus (RSV) expression vectors containing wild-type Tat and either Tat 52/57 or Tat 50/57 (36). The final concentration of the RSV expression vector in each transfection assay was adjusted so that 2.0 μg of this expression vector was present. CAT assays were performed at 48 h posttransfection, and transfection efficiencies were normalized with a cytomegalovirus β-galactosidase expression vec-

* Corresponding author. Mailing address: Division of Molecular Virology, Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-8594. Phone: (214) 648-7570. Fax: (214) 648-8862.



B.

	48									57
Wild-type	G	R	K	K	R	R	E	R	R	R
Tat 52/57	G	R	K	K	G	G	A	G	G	G
Tat 50/57	G	R	G	G	G	G	G	G	G	G

FIG. 1. Construction of transdominant Tat mutant proteins. (A) A schematic of the domain organization of the HIV-1 Tat protein is shown. Triangle, position of the two Tat exons. (B) The amino acids in the wild-type Tat basic region are compared with those present in the Tat 52/57 and Tat 50/57 transdominant mutant proteins which were constructed by site-directed mutagenesis and the mutations were confirmed by DNA sequencing.

tor (36). As can be seen from Fig. 2, wild-type Tat activated HIV-1 gene expression 84-fold (Fig. 2B, lanes 1 and 2), which contrasts with the results with Tat 52/57 and Tat 50/57, which produced no detectable activation of HIV-1 gene expression (Fig. 2B, lanes 3 and 9, respectively). Both transdominant Tat proteins inhibited wild-type Tat activation approximately three- to fourfold after they were added in equimolar amounts with wild-type Tat (Fig. 2B, lanes 4 and 10). Increasing the amount of either Tat transdominant mutant protein inhibited wild-type Tat function in a dose-dependent manner (Fig. 2B, lanes 5 to 8 and 11 to 14). At a 20-fold molar excess of Tat 52/57, wild-type Tat activation was inhibited 12-fold, while Tat 50/57 produced a 40-fold level of inhibition (Fig. 2B, lanes 8 and 14, respectively). This greater inhibition of HIV-1 gene expression by Tat 50/57 was calculated to be 3.7-fold ± 0.6 in three independent experiments with different DNA preparations. These results demonstrated that the Tat 50/57 mutant protein was a strong inhibitor of wild-type Tat activation and that this mutant protein exhibited little ability to transactivate the HIV-1 LTR.

Since H9 cells have been demonstrated to be a highly permissive T-lymphocyte cell line for HIV-1 infection (42), we used these cells to obtain drug-resistant cell lines that expressed either transdominant Tat 50/57 or transdominant Rev M10 or both of these proteins. The Tat 50/57 gene was cloned downstream of the Moloney murine leukemia virus LTR in the pBabe retroviral vector which also contains the puromycin resistance gene (37). The Rev M10 gene was cloned into a cytomegalovirus expression vector which contained the neomycin gene rather than into the pBabe vector because of our finding and that of a previous study that better inhibition of HIV-1 replication was obtained with Rev M10 with the cytomegalovirus promoter (3). Control cell lines were also obtained with pBabe vectors that contained the neomycin or the puromycin drug resistance gene. Drug-selected populations of H9 cells were assayed for the expression of these transdominant mutant proteins by indirect immunofluorescence with polyclonal rabbit antiserum directed against either Tat or Rev and then by confocal microscopy. The rabbit polyclonal Tat antibody to a glutathione *S*-transferase–Tat fusion protein extending between amino acids 1 and 72 was generated, while the Rev polyclonal antibody was obtained from the National Institutes of Health AIDS Reagent Program (ARRP 1436). No significant fluorescence was observed with rabbit polyclonal antibody directed against either Tat or Rev in an uninfected H9 cell line (Fig. 3A and B). The G418-resistant H9 cell line containing Tat 50/57 stained positively in approximately 90% of the cells (Fig. 3C), while Rev M10 was detected in about 70% of the cells (Fig. 3D). The H9 cell line containing both the

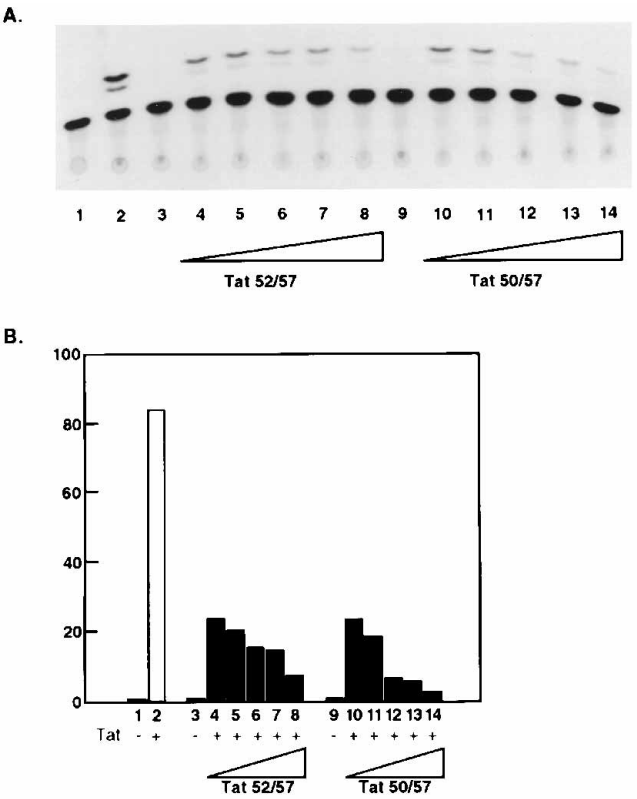


FIG. 2. Comparison of levels of inhibition of wild-type Tat transactivation by transdominant Tat proteins. (A) Transfection of HeLa cells was performed with an HIV-1 LTR CAT reporter in the absence of Tat (lane 1), in the presence of wild-type Tat (lane 2), and in the presence of transdominant Tat mutant proteins Tat 52/57 (lane 3) and Tat 50/57 (lane 9). All Tat expression vectors used in this experiment were cloned downstream of the RSV promoter in the pDp expression vector (40). An expression vector containing either Tat 52/57 (lanes 4 to 8) or Tat 50/57 (lanes 9 to 13) was used at 100 ng (lanes 4 and 10), 200 ng (lanes 5 and 11), 500 ng (lanes 6 and 12), 1 μg (lanes 7 and 13), and 2 μg (lanes 8 and 14) in conjunction with 100 ng of wild-type Tat and 250 ng of the HIV-1 LTR CAT reporter to transfect HeLa cells. The final concentration of the RSV promoter in each transfection was 2.0 μg, and the concentration was adjusted by the addition of the RSV expression vector. CAT assays were performed at 48 h posttransfection. The percent conversions as determined by Phosphorimager quantitation are as follows: 0.2% (lane 1), 17% (lane 2), 0.2% (lane 3), 4.1% (lane 4), 3.5% (lane 5), 2.6% (lane 6), 2.4% (lane 7), 1.4% (lane 8), 0.2% (lane 9), 4.0% (lane 10), 3.1% (lane 11), 1.1% (lane 12), 1.0% (lane 13), and 0.4% (lane 14). (B) A graphic representation of the activation (fold [*y* axis]) of HIV-1 LTR CAT gene expression by Tat relative to basal activity. Lane 1, 1-fold; lane 2, 84-fold; lane 3, 1-fold; lane 4, 22-fold; lane 5, 21-fold; lane 6, 17-fold; lane 7, 13-fold; lane 8, 7-fold; lane 9, 1-fold; lane 10, 22-fold; lane 11, 17-fold; lane 12, 6-fold; lane 13, 5-fold; lane 14, 2-fold. These results are representative of three independent experiments. Transfection efficiencies were normalized with a cytomegalovirus β-galactosidase expression vector.

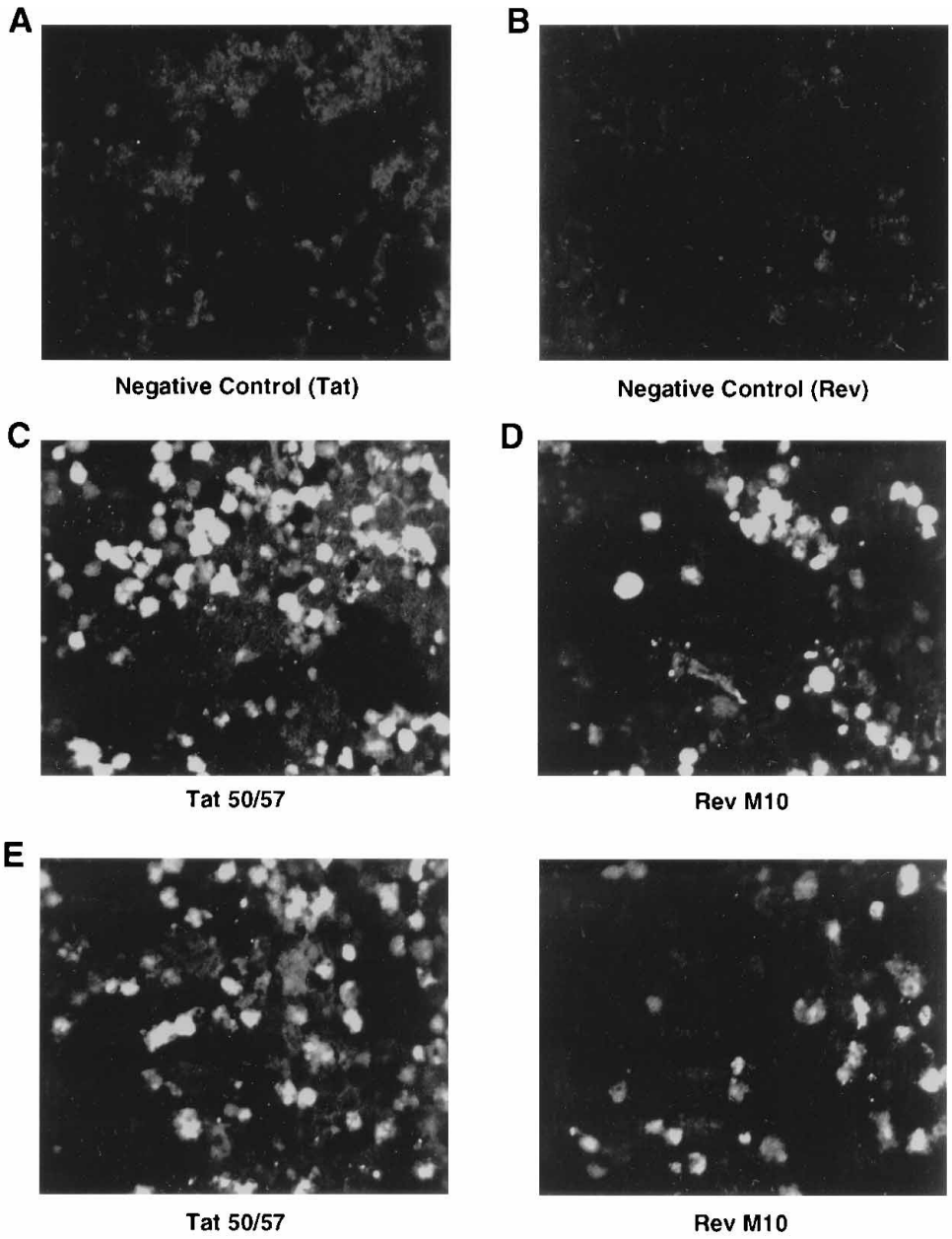


FIG. 3. Indirect immunofluorescence of H9 lines expressing transdominant Tat and Rev proteins. Approximately 10^6 H9 cells expressing either Tat 50/57 or Rev M10 or both these proteins were washed with phosphate-buffered saline, attached to fluorescent antibody slides, and fixed. Rabbit polyclonal serum directed against either Tat (1:100 dilution) (A) or Rev (1:100 dilution of ARRP 1436) (B) was used detect protein expression in H9 cells stably expressing Tat 50/57 (C), Rev M10 (D), or both transdominant proteins (E). The slides were washed, incubated with a 1:300 dilution of fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G, and analyzed by confocal microscopy.

Tat and Rev transdominant proteins expressed these proteins in similar proportions of cells, as can be seen with singly transfected lines (Fig. 3E). The cellular localization of these proteins was difficult to ascertain because of the method of fixation and permeabilization of the H9 cell lines.

In addition, flow cytometry indicated that all cell lines were positive for CD4 staining, with a less than twofold difference between any two cell lines indicating that a reduction in CD4 levels was not responsible for the HIV-1 replication defects (data not shown). Upon being activated with phorbol esters and ionomycin, T cells from different cell lines had levels of interleukin 2 mRNA induction that were similar according to PCR analysis. Also, since Tat has been shown to be involved in apoptosis, we determined whether the transdominant Tat protein could induce apoptosis (13, 18, 24, 50). Chromosomal DNA extracted from each of the cell lines was subjected to agarose gel electrophoresis and then to ethidium staining. No evidence of DNA fragmentation consistent with apoptosis was noted in DNA samples from parental H9 cells or cell lines containing either an individual mutant protein or a combination of transdominant mutant proteins (data not shown).

To determine if the H9 cell lines expressing transdominant Tat or Rev or both experienced inhibited HIV-1 replication kinetics, each cell line was infected with HIV-1 and its repli-

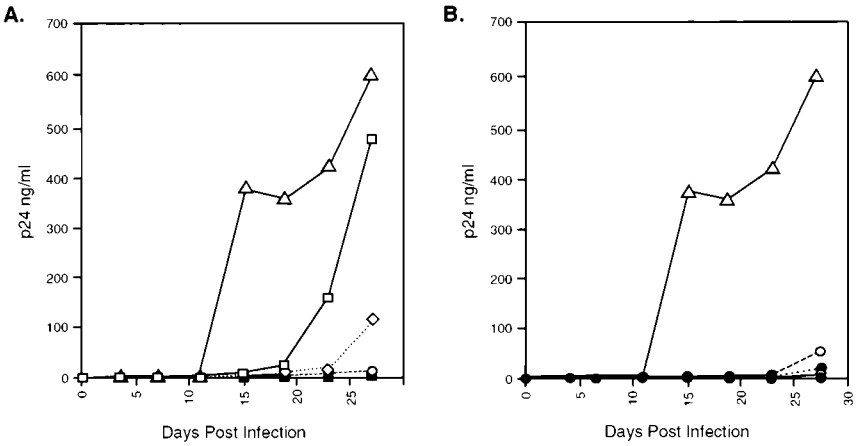


FIG. 4. HIV-1 infection of parental H9 cells and H9 cells expressing transdominant Tat and Rev proteins. (A) Cell lines (5×10^6 cells) were infected with 0.1 50% tissue culture infective dose of the filtered HXB2 strain of HIV-1 per cell and monitored for p24 antigen expression in H9 cells (△); H9 cells expressing Tat 50/57 (□), Rev M10 (◇), and both Tat 50/57 and Rev M10 (○); and mock-infected H9 cells (▩). The cells were split 1:2 every 3 to 4 days for 30 days and maintained in the absence of drug selection. (B) Four other independently transfected H9 cell populations expressing both transdominant Tat and Rev proteins (circles) show a range of inhibition compared with that found in cells with a wild-type infection (△). The p24 antigen levels were determined with a commercial enzyme-linked immunosorbent assay kit from DuPont.

cation was compared with that in the parental H9 cells. H9 cell lines were infected with 0.1 50% tissue culture infective dose of HIV-1 strain IIIB per cell and were maintained in the absence of drug selection during the course of the infection. These HIV-1-infected cells were assayed for p24 antigen every 3 to 4 days for a period of 30 days (Fig. 4). H9 cells containing either the neomycin or the puromycin drug resistance gene or both were positive for p24 antigen within 1 week of infection. HIV-1 replication kinetics in H9 cells expressing Tat 50/57 were delayed compared with those in parental H9 cells by approximately 10 days, after which time the infection rapidly progressed. HIV-1 replication was strongly inhibited in H9 cells that expressed Rev M10, with a delay in replication extending more than 2 weeks postinfection. HIV-1 replication was the most defective in the H9 cell line that expressed both the Tat 50/57 and Rev M10 proteins, with marked inhibition seen for more than 4 weeks postinfection. After 28 days postinfection, parental H9 cells containing the neomycin drug resistance gene alone contained 600 ng of p24 antigen per ml, which was an amount similar to that seen with the Tat 50/57 cell line, while the Rev M10 cell line contained p24 antigen levels that were reduced approximately sixfold. However, the cell line expressing both the Rev and the Tat transdominant mutant proteins contained p24 antigen levels that were reduced approximately 400-fold compared with those for the parental H9 cell line. Four other populations of H9 cells which expressed both the Tat and the Rev transdominant mutant proteins and which were obtained by independent transfection also demonstrated greater inhibition of HIV-1 infection compared with cell lines expressing the single dominant negative mutant proteins (Fig. 4B). In three independent experiments, the presence of Tat 50/57 and Rev M10 resulted in an average 10-fold ± 4-fold decrease in HIV-1 replication compared with that found in the cell line expressing Rev M10 alone at 30 days postinfection. HIV-1 obtained from the H9 cell line expressing the transdominant Tat and Rev proteins gave levels of replication similar to those of the initial HIV-1 viral stocks used to reinfect either parental H9 cells or cell lines containing the transdominant mutants, suggesting that HIV-1 mutants resistant to the transdominant Tat and Rev proteins did not arise (data not shown).

The Rev M10 mutant protein is a potent inhibitor of HIV-1 replication in both T-cell lines and primary cells (3, 5, 28, 30, 49). Regulation of the level of expression of the Rev M10 mutant protein appears to be important for optimal inhibition of HIV-1 replication (26). It has been noted that clonal populations expressing the Rev M10 mutant protein offer the greatest resistance to HIV-1 infection compared with other transdominant mutant proteins, RNA decoys, or ribozymes (3, 49). Previous results with Tat transdominant mutant Δ53 did not show inhibition of HIV-1 replication (3), while another study indicated that cell lines expressing Tat 52/57 showed inhibited HIV-1 replication (1). The defects in HIV-1 replication seen with Tat mutant proteins (10, 15, 45) combined with results demonstrating the inhibition of HIV-1 replication by antisense RNA and polymeric Tat activation response element RNA constructs (25), anti-Tat single-chain intrabodies (35), and anti-Tat ribozymes (27) indicate that inhibition of Tat function can markedly reduce HIV-1 replication. Previously, a fusion of transdominant Tat 52/57 and the Rev M10 mutant protein known as Trev was found to be a better inhibitor of HIV-1 replication than either mutant protein individually (1). However, whether this effect was due to either altered protein stability or localization of the Trev protein was not determined. In the current study, the Tat 50/57 mutant protein inhibited HIV-1 replication, although viral replication did occur after a 10-day delay. The reason for this failure of long-term inhibition was likely due to the fact that at least a three- to fivefold molar excess of the transdominant Tat mutant protein is necessary to markedly inhibit wild-type Tat transactivation. As judged by immunofluorescence staining, not all of the cells expressed high levels of the transdominant Tat mutant protein, because the cells were uncloned populations of drug-resistant cells rather than individual clones of cells which highly expressed Tat 50/57. Therefore, it is likely that the quantities of wild-type Tat synthesized during the HIV-1 infection were sufficient to overcome the effects of the transdominant Tat mutant protein and thus to avoid the inhibition of replication.

The mechanism for transdominant Tat inhibition most likely involves the cytoplasmic sequestration of cellular factors necessary for efficient viral transcription. For instance, a cellular kinase has been demonstrated to bind specifically to the ami-

no-terminal 48 amino acids of HIV-1 Tat and the amino-terminal 81 amino acids of HIV-2 Tat (19). Mutations of critical cysteine residues in Tat prevent the binding of the cellular kinase, and these mutations would also eliminate the effect of the transdominant Tat protein (19, 41). Since this kinase has been demonstrated to phosphorylate the C-terminal domain of RNA polymerase II, the kinase is a candidate for modulating the transcription-activating properties of Tat (19). Thus, the transdominant Tat mutant protein may function by competing with wild-type Tat to prevent the nuclear import of this cellular kinase. The recent demonstration that the effector domain of Rev can interact with a nuclear pore protein (7, 16) suggests that the failure to interact with this protein may be responsible for the inability of the Rev M10 protein to shuttle between the nucleus and the cytoplasm, resulting in the transdominant phenotype. This failure to shuttle between the nucleus and cytoplasm leads to the failure of the transdominant Rev protein to transport nonspliced HIV-1 or single-spliced RNA from the nucleus to the cytoplasm. Given the fact that potential cellular targets for transdominant Tat and Rev proteins can be identified, it is possible that more potent inhibitors of Tat and Rev proteins can be constructed. Since the combination of Tat and Rev mutant proteins has a synergistic effect in preventing HIV-1 replication, these proteins may be a useful combination to test in future gene therapy protocols.

We thank Sharon Johnson and Susan Vaughn for the preparation of the manuscript and D. William Provance, Jr., for assistance with the microscopy studies.

These studies were supported by the NIH and the Department of Veterans Affairs.

REFERENCES

1. **Aguilar-Cordova, E., J. Chinen, L. A. Donehower, J. W. Harper, A. P. Rice, J. S. Butel, and J. W. Belmont.** 1995. Inhibition of HIV-1 by a double transdominant fusion gene. Gene Ther. **2:**181–186.
2. **Ahmed, Y. F., S. M. Hanly, M. H. Malim, B. R. Cullen, and W. C. Greene.** 1990. Structure-function analyses of the HTLV-I Rex and HIV-1 Rev RNA response elements: insights into the mechanism of Rex and Rev action. Genes Dev. **4:**1014–1022.
3. **Bahner, I., C. Zhou, X.-J. Yu, Q.-L. Hao, J. C. Guatelli, and D. B. Kohn.** 1993. Comparison of *trans*-dominant inhibitory mutant human immunodeficiency virus type 1 genes expressed by retroviral vectors in human T lymphocytes. J. Virol. **67:**3199–3207.
4. **Baltimore, D.** 1988. Intracellular immunization. Nature (London) **335:**395–396.
5. **Bevec, D., M. Dobrovnik, J. Hauber, and E. Bohnlein.** 1992. Inhibition of human immunodeficiency virus type 1 replication in human T-cells by retroviral-mediated gene transfer of a dominant-negative Rev trans-activator. Proc. Natl. Acad. Sci. USA **89:**9870–9874.
6. **Bogerd, H., and W. C. Greene.** 1993. Dominant negative mutants of human T-cell leukemia virus type I Rex and human immunodeficiency virus type 1 Rev fail to multimerize in vivo. J. Virol. **67:**2496–2502.
7. **Bogerd, H. P., R. A. Fridell, S. Madore, and B. R. Cullen.** 1995. Identification of a novel cellular cofactor for the Rev/Rex class of retroviral regulatory proteins. Cell **82:**485–494.
8. **Calnan, B. J., S. Biancalana, D. Hudson, and A. D. Frankel.** 1991. Analysis of arginine-rich peptides from the HIV Tat protein reveals unusual features of RNA-protein recognition. Genes Dev. **5:**201–210.
9. **Carroll, R., L. Martarano, and D. Derse.** 1991. Identification of lentivirus Tat functional domains through generation of equine infectious anemia virus/human immunodeficiency virus type 1 *tat* gene chimeras. J. Virol. **65:**3460–3467.
10. **Dayton, A. I., J. G. Sodroski, C. A. Rosen, W. C. Goh, and W. A. Haseltine.** 1986. The trans-activator gene of the human T cell lymphotropic virus type III is required for replication. Cell **44:**941–947.
11. **Dingwall, C., I. Ernberg, M. J. Gait, S. M. Green, S. Heaphy, J. Karn, A. D. Lowe, M. Singh, and M. A. Skinner.** 1990. HIV-1 Tat protein stimulates transcription by binding to a U-rich bulge in the stem of the TAR RNA structure. EMBO J. **9:**4145–4153.
12. **Echetebu, C. O., H. Rhim, C. H. Herrmann, and A. P. Rice.** 1994. Construction and characterization of a potent HIV-2 Tat transdominant mutant protein. J. Acquired Immune Defic. Syndr. **7:**655–664.
13. **Fauci, A. S.** 1993. Multifactorial nature of human immunodeficiency virus disease: implications for therapy. Science **262:**1011–1018.
14. **Feinberg, M. B., R. F. Jarrett, A. Aldovini, R. C. Gallo, and F. Wong-Staal.** 1986. HTLV-III expression and production involve complex regulation at the levels of splicing and translation of RNA. Cell **46:**807–817.
15. **Fisher, A. G., M. B. Feinberg, S. F. Josephs, M. E. Harper, L. M. Marselle, G. Reyes, M. A. Gonda, A. Aldovini, C. Debouk, R. C. Gallo, and F. Wong-Staal.** 1986. The trans-activator gene of HTLV-III is essential for virus replication. Nature (London) **320:**367–371.
16. **Fisher, A. G., J. Huber, W. C. Boeiens, I. W. Mattaj, and R. Luhrmann.** 1995. The HIV-1 Rev activation domain is a nuclear export signal that accesses an export pathway used by specific cellular RNAs. Cell **82:**475–483.
17. **Garcia, J. A., D. Harrich, L. Pearson, R. Mitsuyasu, and R. B. Gaynor.** 1988. Functional domains required for *tat*-induced transcriptional activation of the HIV-1 long terminal repeat. EMBO J. **7:**3143–3147.
18. **Gougeon, M.-L., and L. Montagnier.** 1993. Apoptosis in AIDS. Science **260:**1269–1270.
19. **Herrmann, C. H., and A. P. Rice.** 1995. Lentivirus Tat proteins specifically associate with a cellular protein kinase, TAK, that hyperphosphorylates the carboxyl-terminal domain of the large subunit of RNA polymerase II: candidate for a Tat cofactor. J. Virol. **69:**1612–1620.
20. **Hope, T. J., N. P. Klein, M. E. Elder, and T. G. Parslow.** 1992. Transdominant inhibition of human immunodeficiency virus type 1 Rev occurs through formation of inactive protein complexes. J. Virol. **66:**1849–1855.
21. **Hope, T. J., D. McDonald, X. Huang, J. Low, and T. G. Parslow.** 1990. Mutational analysis of the human immunodeficiency virus type 1 Rev transactivator: essential residues near the amino terminus. J. Virol. **64:**5360–5366.
22. **Kim, S. Y., R. Byrn, J. Groopman, and D. Baltimore.** 1989. Temporal aspects of DNA and RNA synthesis during human immunodeficiency virus infection: evidence for differential gene expression. J. Virol. **63:**3708–3713.
23. **Kubota, S., R. Furuta, M. Maki, and M. Hatanaka.** 1992. Inhibition of human immunodeficiency virus type 1 Rev function by a Rev mutant which interferes with nuclear/nucleolar localization of Rev. J. Virol. **66:**2510–2513.
24. **Li, C. J., D. J. Friendman, C. Wang, V. Meteleve, and A. B. Pardee.** 1995. Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein. Science **268:**429–431.
25. **Lisziewicz, J., D. Sun, B. Trapnell, M. Thomson, H.-K. Chang, B. Ensoli, and B. Peng.** 1995. An autoregulated dual-function *antitat* gene for human immunodeficiency virus type 1 gene therapy. J. Virol. **69:**206–212.
26. **Liu, J., C. Woffendin, Y. Zhi-yong, and G. J. Nabel.** 1994. Regulated expression of a dominant negative form of Rev improves resistance to HIV replication in T-cells. Gene Ther. **1:**32–37.
27. **Lo, K. M. S., M. A. Biasolo, G. Dehni, G. Palu, and W. A. Haseltine.** 1992. Inhibition of replication of HIV-1 by retroviral vectors expressing *tat*-antisense and anti-*tat* ribozyme RNA. Virology **190:**176–183.
28. **Malim, M. H., S. Bohnlein, J. Hauber, and B. R. Cullen.** 1989. Functional dissection of the HIV-1 Rev trans-activator—derivation of a trans-dominant repressor of Rev function. Cell **58:**205–214.
29. **Malim, M. H., and B. R. Cullen.** 1991. HIV-1 structural gene expression requires the binding of multiple Rev monomers to the viral RRE: implications for HIV-1 latency. Cell **65:**241–248.
30. **Malim, M. H., W. W. Freimuth, J. Liu, T. J. Boyle, H. K. Lyerly, B. R. Cullen, and G. J. Nabel.** 1992. Stable expression of transdominant Rev protein in human T-cells inhibits human immunodeficiency virus replication. J. Exp. Med. **176:**1197–1201.
31. **Malim, M. H., J. Hauber, S. Y. Le, J. V. Maizel, and B. R. Cullen.** 1989. The HIV-1 rev trans-activator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. Nature (London) **338:**254–257.
32. **Malim, M. H., D. F. McCarn, L. S. Tiley, and B. R. Cullen.** 1991. Mutational definition of the human immunodeficiency virus type 1 Rev activation domain. J. Virol. **65:**4248–4254.
33. **Malim, M. H., L. S. Tiley, D. F. McCarn, J. R. Rusche, J. Hauber, and B. R. Cullen.** 1990. HIV-1 structural gene expression requires binding of the Rev trans-activator to its RNA target sequence. Cell **60:**675–683.
34. **Meyer, B. E., and M. H. Malim.** 1994. The HIV-1 Rev trans-activator shuttles between the nucleus and the cytoplasm. Genes Dev. **8:**1538–1547.
35. **Mhashilkar, A. M., J. Bagley, S. Y. Chen, A. M. Szilvay, D. G. Helland, and W. A. Marasco.** 1995. Inhibition of HIV-1 Tat-mediated LTR transactivation and HIV-1 infection by anti-Tat single chain intrabodies. EMBO J. **14:**1542–1551.
36. **Modesti, N., J. Garcia, C. Debouck, M. Peterlin, and R. Gaynor.** 1991. Trans-dominant Tat mutants with alterations in the basic domain inhibit HIV-1 gene expression. New Biol. **3:**759–768.
37. **Morgenstern, J. P., and H. Land.** 1990. Advanced mammalian gene transfer: high titre retroviral vectors with multiple drug selection markers and a complementary helper-free packaging cell line. Nucleic Acids Res. **18:**3587–3596.
38. **Nabel, G. J.** 1994. Gene therapy approaches to AIDS. AIDS **8:**561–569.
39. **Olsen, H. S., A. W. Cockrane, P. J. Dillon, C. M. Nalin, and C. A. Rosen.** 1990. Interaction of the human immunodeficiency virus type 1 Rev protein with a structured region in env mRNA is dependent on multimer formation mediated through a basic stretch of amino acids. Genes Dev. **4:**1357–1364.
40. **Olsen, H. S., N. Peter, A. W. Cochrane, and C. A. Rosen.** 1990. Secondary

structure is the major determinant for interaction of HIV Rev protein with RNA. Science **247:**845–848.
41. **Pearson, L., J. Garcia, F. Wu, N. Modesti, J. Nelson, and R. Gaynor.** 1990. A transdominant tat mutant that inhibits tat-induced gene expression from the human immunodeficiency virus long terminal repeat. Proc. Natl. Acad. Sci. USA **87:**5079–5083.
42. **Popovic, M., M. G. Sarnagadheran, E. Recid, and R. C. Gallo.** 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science **224:**497–500.
43. **Roy, S., U. Delling, C.-H. Chen, C. A. Rosen, and N. Sonenberg.** 1990. A bulge structure in HIV-1 TAR RNA is required for Tat binding and Tat-mediated *trans*-activation. Genes Dev. **4:**1365–1373.
44. **Ruben, S., A. Perkins, R. Purcell, K. Joung, R. Sia, R. Burghoff, W. A. Haseltine, and C. A. Rosen.** 1989. Structural and functional characterization of human immunodeficiency virus *tat* protein. J. Virol. **63:**1–8.
45. **Sadaie, M. R., T. Benter, and F. Wong-Staal.** 1988. Site-directed mutagenesis of two trans-regulatory genes (*tat*-III, *trs*) of HIV-1. Science **239:**910–913.
46. **Siomi, H., H. Shida, M. Maki, and M. Hatanaka.** 1990. Effects of a highly basic region of human immunodeficiency virus Tat protein on nucleolar localization. J. Virol. **64:**1803–1807.
47. **Sodroski, J. G., W. C. Rosen, C. Dayton, A. Terwilliger, and W. Haseltine.** 1986. A second post-transcriptional trans-activator gene required for HTLV-III replication. Nature (London) **321:**412–417.
48. **Trono, D., M. Feinberg, and D. Baltimore.** 1989. HIV-1 gag mutants can dominantly interfere with the replication of the wild-type virus. Cell **59:**113–120.
49. **Vandendriessche, T., M. K. L. Chuah, L. Chiang, H.-K. Chang, B. Ensoli, and R. A. Morgan.** 1995. Inhibition of clinical human immunodeficiency virus (HIV) type 1 isolates in primary $CD4^+$ T lymphocytes by retroviral vectors expressing anti-HIV genes. J. Virol. **69:**4045–4052.
50. **Westendorp, M. O., R. Frank, C. Ochsenbauer, K. Stricker, J. Dhein, H. Walczak, K.-M. Debatin, and P. H. Krammer.** 1995. Sensitization of T-cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. Nature (London) **375:**497–500.
51. **Zapp, M. L., T. J. Hope, T. G. Parslow, and M. R. Green.** 1991. Oligomerization and RNA binding domains of the type 1 human immunodeficiency virus Rev protein: a dual function for an arginine-rich binding motif. Proc. Natl. Acad. Sci. USA **88:**7734–7738.