JOURNAL OF VIROLOGY, Dec. 1992, p. 7522–7528
0022-538X/92/127522-07$02.00/0

Vol. 66, No. 12

TAR-Independent Replication of Human Immunodeficiency Virus Type 1 in Glial Cells

OMAR BAGASRA,[1] KAMEL KHALILI,[2] THIKKAVARAPU SESHAMMA,[1] J. PAUL TAYLOR,[2] AND ROGER J. POMERANTZ[1,2]*

The Dorrance H. Hamilton Laboratories, Division of Infectious Disease, Department of Medicine,[1] and Molecular Neurovirology Section, Department of Biochemistry and Molecular Biology, Jefferson Institute of Molecular Medicine,[2] Jefferson Alumni Hall, Thomas Jefferson University, Philadelphia, Pennsylvania 19107-6799

Received 29 June 1992/Accepted 18 August 1992

The molecular mechanisms involved in the replication of human immunodeficiency virus type 1 (HIV-1) may differ in various cell types and with various exogenous stimuli. Astrocytic glial cells, which can support HIV-1 replication in cell cultures and may be infected in vivo, are demonstrated to provide a cellular milieu in which TAR mutant HIV-1 viruses may replicate. Using transfections of various TAR mutant HIV-1 proviral constructs, we demonstrate TAR-independent replication in unstimulated astrocytic cells. We further demonstrate, using viral constructs with mutations in the *tat* gene and in the nuclear factor κB (NF-κB)-binding sites (enhancer) of the HIV-1 long terminal repeat, that TAR-independent HIV-1 replication in astrocytic cells requires both intact NF-κB moiety-binding motifs in the HIV-1 long terminal repeat and Tat expression. We measured HIV-1 p24 antigen production, syncytium formation, and levels and patterns of viral RNA expression by Northern (RNA) blotting to characterize TAR-independent HIV-1 expression in astrocytic glial cells. This alternative regulatory pathway of TAR-independent, Tat-responsive viral production may be important in certain cell types for therapies which seek to perturb Tat-TAR binding as a strategy to interrupt the viral lytic cycle.

Human immunodeficiency virus type 1 (HIV-1) infects a variety of cell types in cell cultures and in vivo (14, 24). Two cell types which harbor HIV-1 in vivo are the CD4-positive T lymphocyte and the monocyte/macrophage (16, 17, 29, 45, 53). Other cell types, including microvascular endothelial cells and astrocytic glial cells, may also be infected with HIV-1 in vivo (29, 41).

Control of HIV-1 replication involves the binding of a variety of HIV-1-encoded proteins and cellular factors, which interact primarily with the long terminal repeat (LTR) on the 5′ end of the provirus (for review, see reference 10). A virus-encoded transactivator of HIV-1 gene expression, Tat, binds to a section of viral RNA transcribed from the R region of the 5′ LTR, TAR, and dramatically stimulates LTR-directed transcription and, possibly, posttranscriptional events (10, 47). The HIV-1 protein Nef has been shown in some studies to downregulate HIV-1 replication, but this is extremely controversial (10). The HIV-1 LTR has a variety of well-described and putative motifs which bind cellular factors and consequently affect transcriptional efficiency. The HIV-1 LTR contains a TATA box; three GC-rich regions, which bind the SP1 transcription factor; and two 11-bp enhancer motifs, which bind nuclear factor κB (NF-κB) (10). Each of these regions has been demonstrated to significantly affect HIV-1 replication (10).

Many of the HIV-1 LTR protein-binding DNA motifs interact differently in various cellular milieus. For example, the three Sp1 sites appear to be necessary for viral replication only if activated NF-κB is not present in a particular intracellular environment (42). NF-κB is important in the response of HIV-1 replication to a variety of exogenous stimuli (30). This transcription factor appears to be a member of a family of proteins related to the *rel* oncogene and has recently been cloned (21, 28, 35). A well-characterized moiety of NF-κB is composed of two proteins, p50 and p65, bound as a heterodimer (35). NF-κB is also bound to a repressor protein, IκB, in the cytoplasm. After stimulation by a variety of agents, including phorbol esters, tumor necrosis factor alpha, and lipopolysaccharide, and following antigenic stimulation, IκB is phosphorylated and dissociates from NF-κB (20, 38). The unbound NF-κB is then transported to the nucleus, where it binds to enhancer regions of a wide variety of viral and cellular genes, including HIV-1. The activation of NF-κB, although important, may not be absolutely essential for HIV-1 replication within all cell types (31).

The virally derived Tat protein, encoded from two exons in multiply spliced HIV-1-specific RNA, has been studied extensively and appears to be intimately tied to high levels of HIV-1 transcription (9, 15, 47). Although Tat appears to increase HIV-1 transcription primarily by affecting elongation and possibly initiation of transcription (9, 15, 47) after binding to the RNA transcribed from the TAR region of the 5′ LTR, in certain systems, a translational effect is also observed (e.g., *Xenopus* oocytes) (5). The TAR RNA has a complex stem-loop structure, with a proximal bulge in the stem (9). Tat appears to bind to this bulge, and the results with Tat fusion proteins (i.e., with other RNA- or DNA-binding proteins) and substitution of a different binding motif for TAR have suggested that TAR functions merely to position Tat close to the 5′ HIV-1 LTR (2, 3, 13, 44, 46, 49).

A number of recent studies suggest that Tat, when bound to TAR, interacts with transcription factors bound to the NF-κB and Sp1 binding motifs (2, 32). Nevertheless, there is also increasing evidence that another cellular factor(s) is necessary for the Tat-TAR effect on viral transcription. Several factors have been demonstrated to bind to TAR

* Corresponding author.

(e.g., a 345-amino-acid factor [18], URBP-1 [19], a 140-kDa TAR-stem binding protein [43], and two factors which bind to the upper portion of the hairpin [TRP1 and TRP2] [48]) and may be functionally important. A particularly well studied 68-kDa cellular factor which binds to the loop of TAR RNA has also been described (33). In an important recent study, a cellular factor (TFIIS) involved in transcriptional elongation could complement the elongation-promoting activity of Tat on the HIV-1 LTR (26). Tat does not appear to function efficiently in murine cells, and this defect can be functionally complemented by human chromosome 12 (37). Protein kinase C has also been demonstrated to be vital for the efficient functioning of Tat (25). Thus, the interaction of the intracellular milieus with Tat function is clearly a key part of the mechanisms involved in the control of HIV-1 expression. Together, these diverse studies strongly point to a cellular cofactor(s) which is critical to Tat function in HIV-1 replication. Cell-specific control of HIV-1 transcription can also be inferred from these data.

To investigate the cell-specific control mechanisms of HIV-1 replication, we have studied HIV-1 expression in astrocytic glial cells, a cell type which may be important in HIV-1-induced central nervous system dysfunction in infected humans (41). Our initial studies of astrocytic glial cells have suggested that TAR-independent expression of chloramphenicol acetyltransferase (CAT) from an HIV-1 LTR promoter occurs in certain astrocytic glial cells (50). Of note, site-directed mutagenesis of TAR in full viral constructs has demonstrated TAR-independent viral replication in the Jurkat T-lymphocyte cell line (23). TAR-independent viral expression occurred only if these T-lymphocytic cells were stimulated with phorbol esters but not in the unstimulated state (23). This TAR-independent viral expression in stimulated Jurkat cells appeared to require intact NF-κB binding sites (enhancer) in the 5′ LTRs of the viral constructs (23). As astrocytic glial cells supported TAR-independent gene expression from an HIV-1 LTR in subgenomic reporter constructs, we evaluated whether unstimulated astrocytic glial cells could support HIV-1 replication from TAR mutant viruses.

As penetration of astrocytic glial cells by HIV-1 may be inefficient (7, 22, 27), we used transfection techniques (1, 38) to efficiently and reproducibly introduce TAR mutant proviruses into astrocytic glial cells as well as other cell lines. The RIP-7 derivative of the HIV-1 clone HXB2 contains an intact TAR sequence and was used as the wild-type virus (34). Three proviral constructs were used in these initial studies. ΔTAR1 was created by partial *Sac*I digestion (single cut) in the viral genome of RIP-7-HXB2 and then blunted with the Klenow fragment of DNA polymerase I, which led to removal of nucleotides +35 to +38 relative to the cap site in the 5′ LTR. The ΔTAR2 mutant was created by a partial *Sac*I digestion that removed all nucleotides between +38 and +187 in the 5′ LTR (kindly provided by Mark Feinberg, Gladstone Institute). The ΔTAR1 mutation disrupted base-pairing in the stem of TAR RNA, while the large deletion mutation in ΔTAR2 completely disrupted the secondary structure of TAR RNA. The ΔTAR3 mutant (kindly provided by David Harrich and Richard Gaynor, University of Texas—Southwestern) was constructed via site-directed mutagenesis of HIV-1 at positions +11 to +14 and +40 to +43 (23). These mutations disrupted base-pairing in the stem of TAR RNA. All plasmids were verified by sequencing to confirm proper construction.

These constructs were transiently transfected into unstimulated and phorbol ester (phorbol 12-myristate 13-acetate [PMA]; Sigma)-stimulated T-lymphocytic cells (H9), a monoblastoid cell line (U937), and U87-MG cells, of glioblastoma origin. The H9 and U937 cells were transfected by a DEAE-dextran method (38), while the astrocytic cells were transfected by a calcium phosphate coprecipitation technique (1). Transfection efficiencies were standardized by cotransfection of a plasmid with the CAT gene downstream of the cytomegalovirus immediate-early promoter and subsequent determination of CAT activity in cellular extracts (39). Virus production was measured by determination of HIV-1 p24 antigen levels in the supernatants with an enzyme-linked immunosorbent assay (ELISA; Du Pont) at 48 h posttransfection.

As illustrated in Fig. 1, unstimulated H9 and U937 cells supported viral replication of the wild-type virus (HXB2) with an intact TAR but not of the three TAR mutant viruses. PMA stimulation of H9 and U937 cells did not affect wild-type viral growth but allowed viral expression by ΔTAR1 in H9 and U937 cells and of ΔTAR3 in H9 but not U937 cells (Fig. 1A and B). ΔTAR2, which contained the most severe disruption of TAR (*Sac*I-*Sac*I deletion), was not viable in either cell line, with or without stimulation by PMA. Interestingly, wild-type HXB2 and all three TAR mutant viruses replicated in astrocytic glial cells (U87-MG) (Fig. 1C). No augmentation in viral expression from any of these HIV-1 proviral constructs was demonstrated in U87-MG cells after PMA stimulation (Fig. 1C). This pattern of TAR-independent viral replication was also demonstrated in a second unstimulated astrocytic glial cell line of glioblastoma origin (U138-MG) (data not illustrated). Of note, HIV-1 p24 antigen production in the astrocytic glial cell lines transfected with wild-type and TAR mutant viruses peaked at 48 h posttransfection and then plateaued over 10 days posttransfection. A continued rise in HIV-1 p24 antigen levels occurred over the 10 days posttransfection in the H9 and U937 cells transfected with wild-type virus (data not shown). The early plateau of HIV-1 p24 antigen levels in transfected astrocytic glial cells may be secondary to poor virus spread, as demonstrated previously (7, 22, 27) in cultures of these cells. These data therefore demonstrate Tar-independent HIV-1 replication in unstimulated astrocytic glial cells.

To further characterize TAR-independent viral replication in unstimulated glial cells, the patterns of HIV-1-specific RNA expression were evaluated in these transfections by Northern blot hybridization (40). Wild-type expression of viral RNA, as characterized by nearly equivalent levels of unspliced (9.2-kb), singly spliced (4.3-kb), and multiply spliced (2-kb) HIV-1-specific RNA species (40), was demonstrated in unstimulated H9 and U87-MG cells (Fig. 2). Wild-type patterns of HIV-1-specific RNA expression were also demonstrated in unstimulated astrocytic glial cells transfected with ΔTAR1 or ΔTAR2 (Fig. 2). No viral RNA expression was demonstrated in unstimulated H9 cells transfected with ΔTAR1 or ΔTAR2 (Fig. 2). Importantly, an aberrant pattern of viral RNA expression characterized by very low or undetectable levels of unspliced HIV-1-specific RNA with significant levels of multiply spliced viral RNA species, a pattern which has been demonstrated in certain latently infected cell types (40), was not present in these transfections of H9 and astrocytic glial cells.

Additional proviral constructs were then used in transfection experiments to further evaluate TAR-independent HIV-1 replication in unstimulated astrocytic cells. As previous work had demonstrated that TAR-independent replication in PMA-stimulated T-lymphocytic cells required an intact enhancer element (NF-κB binding sites) in the HIV-1

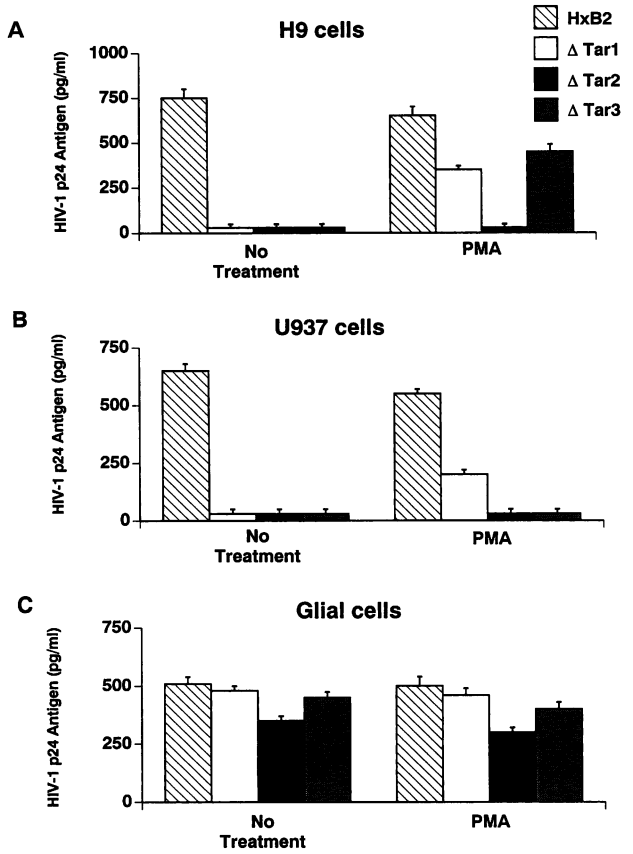

FIG. 1. Transient transfections of HIV-1 constructs into various cell lines. HIV-1 constructs (10 μg) were transfected into (A) H9 cells (lymphocytic), (B) U937 cells (monoblastoid), and (C) astrocytic glial cells (U87-MG). HIV-1 p24 antigen levels in the supernatants were measured 48 h posttransfection by ELISA. Ten million H9 and U937 cells, at an initial concentration of 2×10^5 cells per ml, were used in each transfection, while the glial cells were transfected in 100-mm petri dishes when they were 40% confluent. The H9 and U937 cells were maintained in RPMI 1640 medium with 10% fetal calf serum and transfected by a DEAE-dextran method described previously (38). The astrocytic cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum (GIBCO) and transfected by a calcium phosphate coprecipitation method (1). The transfection efficiencies were normalized by cotransfection of a construct containing the CAT gene downstream of the cytomegalovirus immediate-early promoter (CMV-CAT) (2 μg) (39). CAT activity was then measured in cellular extracts after the protein contents of the cell extracts were measured and adjusted (39). Some transfection mixtures were left untreated, while others were treated with PMA (50 ng/ml) (Sigma) at 24 h posttransfection. In each experiment, transfections with a plasmid without HIV-1 sequences, pSP65 (Promega), were done as negative controls. The data in these graphs represent the arithmetic means of at least three independent experiments performed in duplicate with two different plasmid preparations ± standard deviations.

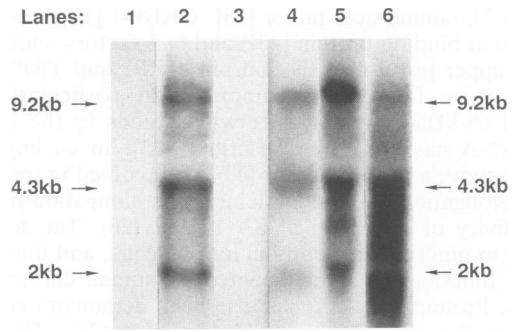

FIG. 2. Northern blot analyses of transient transfections. Total cellular RNA was harvested 48 h posttransfection by an acid guanidinium method as previously described (8). The RNA samples were then treated with RNase-free DNase (20 μg) (Promega) in 10 mM Tris (pH 7.4)–10 mM $MgCl_2$ for 1 h at 37°C to degrade residual transfected plasmid. Equal quantities of total cellular RNA (20 μg) were electrophoresed on a 1.1% agarose–formaldehyde gel, transferred to nitrocellulose, and hybridized to a [^{32}P]RNA probe at 60°C overnight. The RNA probe was generated with T7 RNA polymerase (Boehringer Mannheim) and purified on a Quick Spin Q-50 Sephadex column (Boehringer Mannheim). The probe is complementary to nucleotides 8475 to 8900 of the HIV-1 genome and binds to all HIV-1-specific RNA species. Filters were vigorously washed in 0.2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 68°C and exposed to X-ray film (40). These autoradiographs are representative of at least two independent experiments. Lane 1, ΔTAR1 in unstimulated H9 cells; lane 2, ΔTAR1 in unstimulated glial cells (U87-MG); lane 3, ΔTAR2 in H9 cells; lane 4, ΔTAR2 in U87-MG cells; lane 5, HXB2 in H9 cells; lane 6, HXB2 in U87-MG cells.

LTR (23), we used a plasmid with a wild-type viral backbone and mutations in both NF-κB binding sites, which inhibit activated NF-κB binding to these motifs (enhancer mutant) (23). In addition, the ΔTAR3 plasmid, also containing the above-described site-directed mutations in the enhancer elements (enhancer-plus ΔTAR3 mutant), was used (kindly provided by Richard Gaynor and David Harrich) (23). As illustrated in Fig. 3, the enhancer mutant virus with an intact TAR replicated to significant but lower than wild-type levels in H9 and U937 cells upon transfection (compare Fig. 1A and B with 3A and B). Although the enhancer mutant virus replicated in astrocytic glial cells (U87-MG), the levels of HIV-1 p24 antigen detected in the supernatants were three- to fourfold lower than with the wild-type virus in U87-MG cells (compare Fig. 3C and Fig. 1C). No augmentation in viral expression of the enhancer mutant was noted in any cell line with PMA treatment (Fig. 3). Interestingly, the proviral construct with mutations in both TAR (ΔTAR3 mutation) and the enhancer elements did not replicate in unstimulated or stimulated astrocytic glial cells (Fig. 3C). Thus, intact enhancer sites are required for TAR-independent HIV-1 replication in astrocytic cells.

To determine whether the virally encoded protein Tat was necessary for TAR-dependent and TAR-independent HIV-1 replication, a proviral construct which does not express functional Tat because of the insertion of an *Mlu*I linker in the first exon of *tat* (*Bsu* 361 site) (15) but with an intact TAR region was used for transfection experiments. This Tat mutation was also introduced into the ΔTAR1 and ΔTAR2 constructs by replacing the 3′ proviral end of the ΔTAR1 and ΔTAR2 constructs (*Sal*I site [nucleotide 5785] to an *Xba*I site [in the flanking sequences]) with the 3′ proviral portion from the Tat mutant provirus. No viral replication was demonstrated in unstimulated or stimulated H9, U937, or U87-MG cells after transfection of the Tat mutant proviral constructs (with or without intact TAR regions) (Fig. 4 and data not shown).

To further evaluate the requirements for Tat in TAR-independent replication within astrocytic glial cells, the Tat mutant virus with an intact TAR region and the ΔTAR1 and ΔTAR2 constructs, with the Tat mutation in each construct,

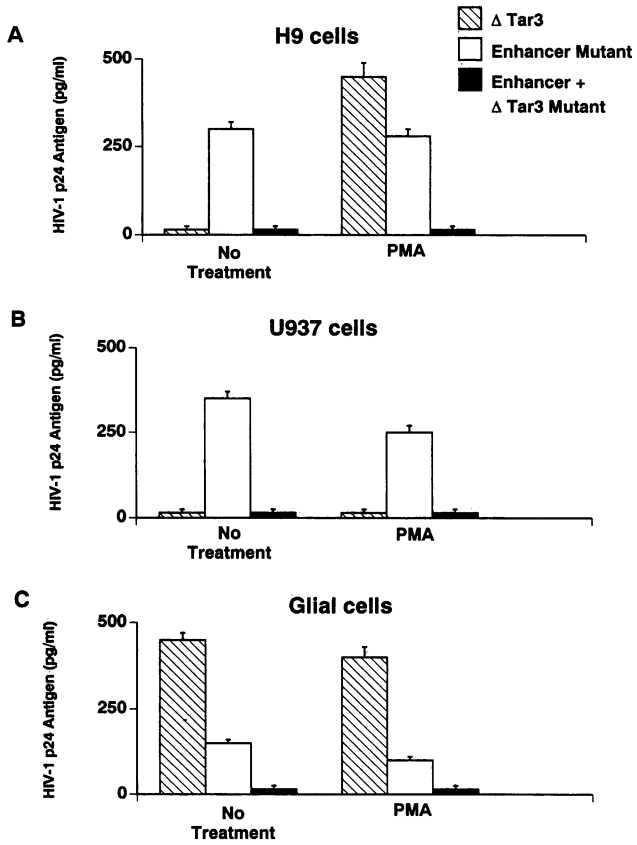

FIG. 3. Transient transfections of enhancer-negative mutant HIV-1 constructs. Various cell lines were transiently transfected with viral constructs (10 μg) (see Fig. 1 legend) containing mutations in TAR (ΔTAR3), with or without mutations in the NF-κB binding sites, as well as a construct with an intact TAR but mutated NF-κB binding sites (enhancer). Some transfection mixtures were treated with PMA (50 ng/ml) at 24 h posttransfection. Supernatants were harvested at 48 h posttransfection for measurement of HIV-1 p24 antigen levels by ELISA. (A) H9 cells; (B) U937 cells; (C) astrocytic glial cells (U87-MG). The data represent the arithmetic means of at least three independent experiments performed in duplicate ± standard deviations.

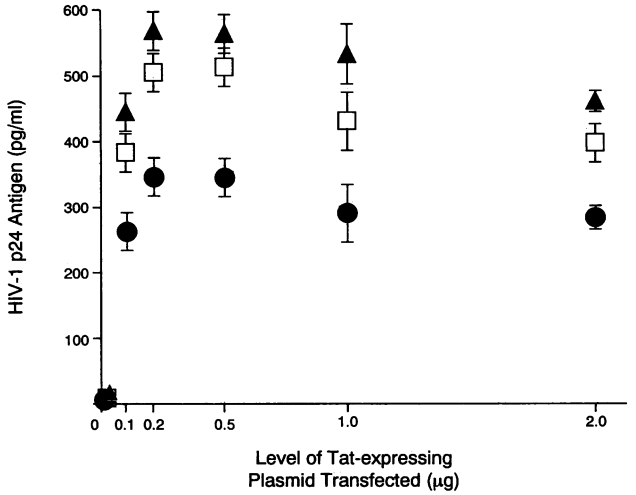

FIG. 4. Effects of Tat on TAR-independent replication in astrocytic glial cells. U87-MG cells were transiently transfected with the Tat mutant provirus with an intact TAR region (▲) and the ΔTAR1 (□) and ΔTAR2 (●) constructs, also containing mutant *tat* genes (10 μg). Various quantities of a Tat-expressing plasmid (pSV-Tat) were cotransfected (36). The data represent the arithmetic means of at least two independent experiments performed in duplicate ± standard deviations.

were transfected into U87-MG cells with various quantities of a Tat-expressing plasmid, pSV-Tat, which contains the first exon of *tat* downstream of a simian virus 40 promoter (36). Increasing levels of viral replication were demonstrated with each proviral construct as increasing quantities of this Tat-expressing plasmid were added in cotransfection experiments with a standard quantity of the proviral constructs (Fig. 4). Of note, an upper limit of and gradual decrease in viral production were demonstrated with increasing quantities of pSV-Tat with all proviral constructs evaluated (Fig. 4). This plateau and possible biphasic effect of Tat in TAR-independent replication, as noted previously for TAR-dependent HIV-1 replication (15), will require further analyses. Therefore, Tat is demonstrated to be necessary for viral replication in these cells whether or not a functional TAR region is present in the virus.

Finally, as a further dramatic demonstration of TAR-independent HIV-1 replication in unstimulated astrocytic glial cells, transfectants of U87-MG cells were fixed, stained, and evaluated for HIV-1-induced syncytium formation at 48 h posttransfection (Fig. 5). Significant numbers of multinucleated giant cells were observed after transfection of the three ΔTAR mutants and HXB2 (Fig. 5). The syncytia were of the same approximate size and character in the TAR mutant and in the wild-type transfections. No syncytia were noted in transfections in which a plasmid without HIV-1 sequences was used (Fig. 5). Fewer syncytia were observed in transfections with the enhancer mutant virus, and no syncytia were demonstrated in the transfections with the proviral construct with mutations in both TAR (ΔTAR3) and the enhancer motifs (Fig. 5).

These data demonstrate that TAR-independent HIV-1 replication is supported in certain cell types. Thus, TAR-independent HIV-1 replication is now demonstrated to occur within cells in a baseline state, unperturbed by exogenous stimulatory factors. This TAR-independent viral expression in astrocytic glial cells, as in PMA-stimulated T lymphocytes (23), requires an intact enhancer element in the LTR. Interestingly, unique cellular factors, not demonstrated in stimulated T lymphocytes, which bind to the HIV-1 enhancer motifs have been recently demonstrated in unstimulated astrocytic glial cells (50). Further studies on how Tat stimulates the replication of HIV-1 in the absence of TAR may also shed light on the mechanisms of Tat function and on possible cell-specific factors which may functionally complement TAR. Our data suggests that cellular factors found in specific intracellular milieus may obviate the requirement for TAR to allow Tat-induced transactivation. Further investigations of the molecular mechanisms underlying TAR-independent HIV-1 expression are on-going in our laboratories.

Astrocytic glial cell lines have been studied in vitro in relation to HIV-1 infection, as their role in HIV-1-induced central nervous system dysfunction remains controversial (41, 53). A variety of astrocytic glial cell lines have been infected with HIV-1 in cell culture (6, 7, 11). Although some have been demonstrated to allow highly productive infection (12), many produce low levels of HIV-1 and may be latently

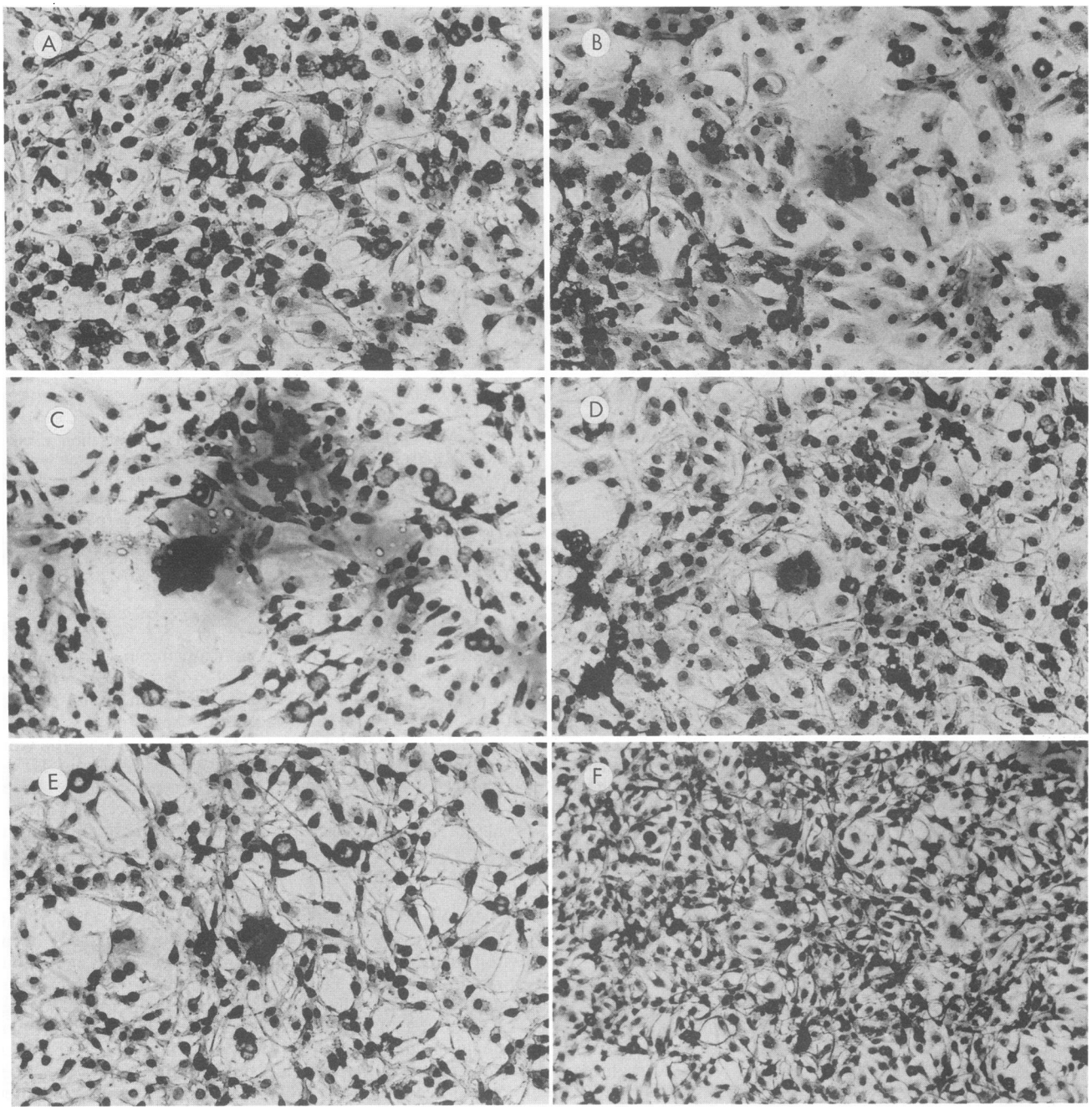

FIG. 5. Syncytium formation in astrocytic glial cells. At 72 h posttransfection, astrocytic glial cells (U87-MG) were washed with phosphate-buffered saline, fixed in absolute methanol, and stained with the Giemsa-Wright method. Syncytium formation was evaluated with an optical microscope. (A) pSP65 (without HIV-1 sequences); (B) HXB2; (C) ΔTAR1; (D) ΔTAR2; (E) ΔTAR3; (F) ΔTAR3 with enhancer mutations. Magnification, ×115.

infected (27). The concept of proviral latency in astrocytic glial cells is complex. Human fetal glial cell explants have been demonstrated to pass through an early phase of productive HIV-1 infection and then remain in a state of low-level persistent infection (51). In a preliminary study, it was suggested that latent HIV-1 infection in astrocytic cells may be secondary to increased expression of Nef (4). Nevertheless, in an important recent study, stable CD4 antigen transfections into neuroglioma cells allowed high levels of HIV-1 expression in these cells (52). Thus, at least in some astrocytic lines, the spread and penetration of the virus within a culture may be a primary determinant of viral production.

If TAR-independent HIV-1 replication occurs in specific primary cells in infected individuals, this may have profound implications for the development of new therapies designed to combat clinical HIV-1 infection (14). Further studies will be required to determine whether TAR-independent HIV-1

expression in vivo could circumvent therapies which might target Tat-TAR interactions.

We thank Mark Feinberg, Richard Gaynor, and David Harrich for providing some of the viral constructs used in these studies and for helpful discussions and Rita M. Victor for excellent secretarial assistance.

This work was supported in part by Public Health Service grants AI00930 (R.J.P.) and AI31836 (R.J.P.) and by a grant from the W. W. Smith Charitable Trust (R.J.P.).

REFERENCES

1. **Ausubel, F., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1989. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
2. **Berkhout, B., A. Gatignol, A. B. Rabson, and K. T. Jeang.** 1990. TAR-independent activation of the HIV-1 LTR: evidence that Tat requires specific regions of the promoter. Cell **62**:757–767.
3. **Berkhout, B., and K. T. Jeang.** 1992. Functional roles for the TATA promoter and enhancers in basal and Tat-induced expression of the human immunodeficiency virus type 1 long terminal repeat. J. Virol. **66**:139–149.
4. **Brack-Werner, R., A. Kleinschmidt, A. Ludvigsen, W. Mellert, M. Neumann, R. Herrmann, M. C. L. Khim, A. Burny, N. Muller-Lantzsch, D. Stavrou, and V. Erfle.** 1992. Infection of human brain cells by HIV-1: restricted virus production in chronically infected human glial cell lines. AIDS **6**:273–285.
5. **Braddock, M., A. M. Thorburn, A. J. Kingsman, and S. M. Kingsman.** 1991. Blocking of Tat-dependent HIV-1 RNA modification by an inhibitor of RNA polymerase II processivity. Nature (London) **350**:439–441.
6. **Cheng-Mayer, C., J. T. Rutka, M. L. Rosenblum, T. McHugh, D. P. Stites, and J. A. Levy.** 1987. Human immunodeficiency virus can productively infect cultured human glial cells. Proc. Natl. Acad. Sci. USA **84**:3526–3530.
7. **Chiodi, F., S. Fuerstenberg, M. Gidlund, B. Asjo, and E. M. Fenyo.** 1987. Infection of brain-derived cells with the human immunodeficiency virus. J. Virol. **61**:1244–1247.
8. **Chomczynski, P., and N. Sacchi.** 1987. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162**:156–157.
9. **Cullen, B. R.** 1990. The HIV-1 Tat protein: an RNA sequence-specific processivity factor? Cell **63**:655–657.
10. **Cullen, B. R., and W. C. Greene.** 1989. Regulatory pathways governing HIV-1 replication. Cell **58**:423–426.
11. **Dewhurst, S., K. Sakai, J. Bresser, M. Stevenson, M. J. Evinger-Hodges, and D. J. Volsky.** 1987. Persistent productive infection of human glial cells by human immunodeficiency virus (HIV) and by infectious molecular clones of HIV. J. Virol. **61**:3774–3782.
12. **Dewhurst, S., K. Sakai, X. H. Zhang, A. Wasiak, and D. J. Volsky.** 1988. Establishment of human glial cell lines chronically infected with the human immunodeficiency virus. Virology **62**:151–159.
13. **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 stimulated transcription by binding to a U-rich bulge in the stem of the TAR RNA structure. EMBO J. **9**:4145–4153.
14. **Fauci, A. S.** 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. Science **239**:617–622.
15. **Feinberg, M. B., D. Baltimore, and A. D. Frankel.** 1991. The role of Tat in the human immunodeficiency virus life cycle indicates a primary effect on transcriptional elongation. Proc. Natl. Acad. Sci. USA **88**:4045–4049.
16. **Gabuzda, D. H., D. D. Ho, S. M. de la Monte, M. S. Hirsch, T. R. Rota, and R. A. Sobel.** 1986. Immunohistochemical identification of HTLV-III antigen in brains of patients with AIDS. Ann. Neurol. **20**:289–295.
17. **Gartner, S., P. Markovits, D. Z. Markovitz, M. H. Kaplan, R. C. Gallo, and M. Popovic.** 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. Science **233**:214–219.
18. **Gatignol, A., A. Buckler-White, B. Berkhout, and K. T. Jeang.** 1991. Characterization of a human TAR RNA-binding protein that activates the HIV-1 LTR. Science **251**:1597–1599.
19. **Gaynor, B., E. Soultanakis, M. Kuwabara, J. Garcia, and D. S. Sigman.** 1989. Specific binding of a HeLa cell nuclear protein to RNA sequences in the human immunodeficiency virus transactivating region. Proc. Natl. Acad. Sci. USA **86**:4858–4862.
20. **Ghosh, S., and D. Baltimore.** 1990. Activation *in vitro* of NF-κB by phosphorylation of its inhibitor, IκB. Nature (London) **344**:678–682.
21. **Ghosh, S., A. M. Gifford, L. R. Riviere, P. Tempst, G. P. Nolan, and D. Baltimore.** 1990. Cloning of the p50 DNA binding subunit of NF-κB: homolog to rel and dorsal. Cell **62**:1019–1029.
22. **Harouse, J. M., S. Bhat, S. L. Spitalnik, M. Laughlin, K. Stefano, D. H. Silberberg, and F. Gonzalez-Scarano.** 1991. Inhibition of entry of HIV-1 in neural cell lines by antibodies against galactosylceramide. Science **253**:320–323.
23. **Harrich, D., J. Garcia, R. Mitsuyasu, and R. Gaynor.** 1990. TAR independent activation of the human immunodeficiency virus in phorbol ester stimulated T lymphocytes. EMBO J. **9**:4417–4423.
24. **Ho, D. D., R. J. Pomerantz, and J. C. Kaplan.** 1987. Pathogenesis of infection with human immunodeficiency virus. N. Engl. J. Med. **317**:278–286.
25. **Jakobovits, A., R. Rosenthal, and D. J. Capon.** 1990. Transactivation of HIV-1 LTR-directed gene expression by Tat requires protein kinase. EMBO J. **9**:1165–1170.
26. **Kato, H., H. Sumimoto, P. Pognonec, C.-H. Chen, C. A. Rosen, and R. G. Roeder.** 1992. HIV-1 Tat acts as a processivity factor in vitro in conjunction with cellular elongation factors. Genes Dev. **6**:655–666.
27. **Keys, B., J. Albert, J. Kovamees, and F. Chiodi.** 1991. Brain-derived cells can be infected with HIV-1 isolates derived from both blood and brain. Virology **183**:834–839.
28. **Kieran, M., V. Blank, F. Logeat, J. Vandekerckhove, F. Lottspeich, O. LeBail, M. B. Urban, P. Kourilsky, P. A. Baeurle, and A. Israel.** 1990. The DNA binding subunit of NF-κB is identical to factor KBF1 and homologous to the rel oncogene product. Cell **62**:1007–1018.
29. **Koenig, S., H. E. Gendelman, J. M. Orenstein, M. C. Dal Canto, G. H. Pezeshkpour, M. Yungbluth, F. Janotta, A. Aksamit, M. A. Martin, and A. S. Fauci.** 1986. Detection of AIDS virus in macrophages in brain tissue from AIDS patients. Science **233**:1089–1093.
30. **Lenardo, M. J., and D. Baltimore.** 1989. NF-κB: a pleiotropic mediator of inducible and tissue-specific gene control. Cell **58**:227–229.
31. **Leonard, J., C. Parrott, A. J. Buckler-White, W. Turner, E. K. Ross, M. A. Martin, and A. B. Rabson.** 1989. The NF-κB binding sites in the human immunodeficiency virus type 1 long terminal repeat are not required for viral infectivity. J. Virol. **63**:4919–4924.
32. **Liu, J., N. D. Perkins, R. M. Schmid, and G. J. Nabel.** 1992. Specific NF-κB subunits act in concert with Tat to stimulate human immunodeficiency virus type 1 transcription. J. Virol. **66**:3883–3887.
33. **Marciniak, R. A., M. A. Garcia-Blanco, and P. A. Sharp.** 1990. Identification and characterization of a HeLa nuclear protein that specifically binds to the transactivation-response (TAR) element of human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **87**:3624–3628.
34. **McCune, J. M., L. B. Rubin, M. B. Feinberg, M. Liberman, J. C. Rosek, G. R. Reyes, and I. L. Weissman.** 1988. Endoproteolytic cleavage of gp160 is required for activation of HIV. Cell **53**:55–67.
35. **Molitor, J. A., W. H. Walker, S. Doerre, D. W. Ballard, and W. C. Greene.** 1990. NF-κB: a family of inducible and differentially expressed enhancer-binding proteins in human T cells. Proc. Natl. Acad. Sci. USA **82**:10028–10032.
36. **Muesing, M., D. H. Smith, and D. J. Capon.** 1987. Regulation of mRNA accumulation by a HIV transactivator protein. Cell **48**:671–701.
37. **Newstein, M., E. J. Stanbridge, G. Casey, and P. R. Shank.** 1990. Human chromosome 12 encodes a species-specific factor which increases human immunodeficiency virus type 1 Tat-mediated transactivation in rodent cells. J. Virol. **64**:4565–4567.

38. **Pomerantz, R. J., M. B. Feinberg, D. Trono, and D. Baltimore.** 1990. Lipopolysaccharide is a potent monocyte/macrophage-specific stimulator of human immunodeficiency virus type 1 expression. J. Exp. Med. **172:**253–261.
39. **Pomerantz, R. J., T. Seshamma, and D. Trono.** 1992. Efficient replication of human immunodeficiency virus type 1 requires a threshold level of Rev: potential implications for latency. J. Virol. **66:**1809–1813.
40. **Pomerantz, R. J., D. Trono, M. B. Feinberg, and D. Baltimore.** 1990. Cells nonproductively infected with HIV-1 exhibit an aberrant pattern of viral RNA expression: a molecular model for latency. Cell **62:**1271–1276.
41. **Price, R. W., B. Brew, J. Sidtis, M. Rosenblum, A. C. Scheck, and P. Cleary.** 1988. The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. Science **239:**586–592.
42. **Ross, E. K., A. J. Buckler-White, A. B. Rabson, G. Englund, and M. A. Martin.** 1991. Contribution of NF-κB and Sp1 binding motifs to the replicative capacity of human immunodeficiency virus type 1: distinct patterns of viral growth are determined by T-cell types. J. Virol. **65:**4350–4358.
43. **Rounseville, M. P., and A. Kumar.** 1992. Binding of a host cell nuclear protein to the stem region of human immunodeficiency virus type 1 *trans*-activation-responsive RNA. J. Virol. **66:** 1688–1694.
44. **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 transactivation. Genes Dev. **4:**1365–1373.
45. **Schnittman, S. M., M. C. Psallidopoulos, H. C. Lane, L. Thompson, M. Baseler, F. Massari, C. H. Fox, N. P. Salzman, and A. S. Fauci.** 1989. The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. Science **245:**305–308.
46. **Selby, M. J., and B. M. Peterlin.** 1990. Transactivation by HIV-1 Tat via a heterologous RNA binding protein. Cell **62:**769–776.
47. **Sharp, P. A., and R. A. Marciniak.** 1989. HIV TAR: an RNA enhancer? Cell **59:**229–230.
48. **Sheline, C. T., L. H. Milocco, and K. A. Jones.** 1991. Two distinct nuclear transcription factors recognize loop and bulge residues of the HIV-1 TAR RNA hairpin. Genes Dev. **5:**2508–2520.
49. **Southgate, C., M. L. Zapp, and M. R. Green.** 1990. Activation of transcription by HIV-1 Tat protein tethered to nascent RNA through another protein. Nature (London) **345:**640–642.
50. **Taylor, J. P., R. J. Pomerantz, O. Bagasra, M. Chowdury, J. Rappaport, K. Khalili, and S. Amini.** 1992. TAR-independent transactivation by Tat in cells derived from the CNS: a novel mechanism of HIV-1 gene regulation. EMBO J. **11:**3395–3403.
51. **Tornatore, C., A. Nath, K. Amemiya, and E. O. Major.** 1991. Persistent human immunodeficiency virus type 1 infection in human fetal glial cells reactivated by T-cell factor(s) or by the cytokines tumor necrosis factor alpha and interleukin-1 beta. J. Virol. **65:**6094–6100.
52. **Volsky, B., K. Sakai, M. M. Reddy, and D. J. Volsky.** 1992. A system for the high efficiency replication of HIV-1 in neural cells and its application to anti-viral evaluation. Virology **186:**303–308.
53. **Watkins, B. A., H. H. Dorn, W. B. Kelly, R. C. Armstrong, B. J. Potts, F. Michaels, C. V. Kufta, and M. Dubois-Dalcq.** 1990. Specific tropism of HIV-1 for microglial cells in primary human brain cultures. Science **249:**549–554.