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Activation of the Human Immunodeficiency Virus by Herpes Simplex Virus Type 1

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Herpes simplex virus type 1 (HSV-1) and some of its immediate-early genes stimulate expression of the human immunodeficiency virus (HIV) long terminal repeat (LTR) sequences and the replication of HIV itself. To demonstrate this, the HIV LTR was linked to the indicator gene chloramphenicol acetyltransferase (CAT) and transfected into Vero cells with or without the *trans*-activating gene (*tat*) of HIV. Infection of these cells with HSV-1 strain KOS or temperature-sensitive mutant *ts*B21 or *ts*E6 resulted in a large increase in CAT activity in the absence of *tat* and further augmentation in the presence of *tat*. This stimulation was seen at both their permissive (34°C) and nonpermissive (39°C) temperatures, implying either that HSV-1 infection or immediate-early gene expression is all that is required. In cotransfection assays in Vero cells, cloned HSV-1 immediate-early genes ICP0 and ICP4 stimulated CAT activity in the presence of *tat*, while ICP27 had no effect. On the other hand, in SW480 cells, ICP4 and, to a lesser extent, ICP0 genes caused stimulation of CAT activity in the absence of *tat*. Deletion mutants within the HIV LTR showed that the target for HSV stimulation is distinct from the *tat*-responsive area and maps near the SP1 binding sites. In HeLa cells, ICP0 or ICP4 stimulated the replication of a cotransfected clone of HIV, as shown by an increase in reverse transcriptase activity in the culture supernatant.

The human immunodeficiency virus (HIV), also known as human T-lymphotropic virus type III or lymphadenopathy-associated virus, is a retrovirus that causes the acquired immune deficiency syndrome (2, 9, 22). Infected individuals often do not develop overt signs or symptoms of disease for many months to years (24). During this latent or disease-free phase, low numbers of HIV-infected cells (1 in 10^4 to 10^6 infected lymphocytes) can be detected by in situ hybridization (15; Koenig and Gendelman, unpublished data). The mechanisms involved in maintenance of this low level of virus expression are not understood but may, in part, involve interactions between cellular and viral regulatory elements. Elucidation of these mechanisms could help determine why HIV remains quiescent in some persons but leads to fatal disease in others.

The regulation of gene expression in HIV is complex. Transcription of viral mRNAs and progeny virion RNA originates from the viral long terminal repeat (LTR) sequences. The HIV LTR contains transcriptional regulatory sequences present in many eucaryotic and viral promoters and includes a TATA box, three SP1 binding sites (7), a core enhancer region, and a negative regulatory region (17, 19, 23). HIV also encodes a *trans*-activating protein (*tat*) whose target is in an area of the HIV LTR known as TAR, which maps between −17 and +80 bases relative to the cap site (34). *tat* exhibits multiple activities, including transcriptional activation and posttranscriptional enhancement of the expression of HIV mRNA (6, 34, 35, 38, 40, 41). An additional regulatory gene, the antirepression *trans*-activator, is involved in posttranscriptional events (34).

Various stimuli, both infectious (viral infections and bacterial products) and immunologic (mitogens and growth factors), stimulate expression of HIV in cultured lymphocytes and monocytes (10, 14, 42). We and others previously reported that herpes simplex virus (HSV) and other DNA virus sequences can stimulate expression of the HIV LTR (10, 25). HSV, a ubiquitous human pathogen that causes recurrent fever blisters, genital lesions, and other severe infections, replicates in a large number of human cell types, including human peripheral blood lymphocytes (28, 32). HSV replication is highly regulated, with three classes of genes expressed at different stages (16). The immediate-early (alpha) genes (ICP0, ICP4, ICP22, ICP27, and ICP47) appear to be regulatory in nature. Their transcription is dependent on host cell factors but can be stimulated by a component of the infecting virion. The proteins ICP0 (Vmw110) and ICP4 (Vmw175) *trans*-activate genes of the early (beta) and late (gamma) classes (8, 17, 29, 30). ICP27 (Vmw63), on the other hand, appears to be involved in regulation of late protein synthesis (36).

In this paper, we demonstrate that HSV infection of cells previously transfected with HIV LTR-*cat* can cause over-100-fold stimulation in chloramphenicol acetyltransferase (CAT) activity. In the presence of *tat*, further activation of the HIV LTR is seen. Experiments using a series of deletion mutants in the HIV LTR showed that the target for HSV activation maps near the SP1 binding sites and not the TAR region. In addition, we have shown that HSV immediate-early genes ICP0 and ICP4 can stimulate activation of HIV LTR-*cat*, as well as production of HIV reverse transcriptase activity. These effects vary in different cell lines, implying that certain cellular factors, in addition to the immediate-early genes of HSV, are involved.

MATERIALS AND METHODS

Cells and viruses. Vero cells and low-passage HeLa-JW cells (a gift of Barrie Carter) were grown in a 1:1 mixture of minimal essential medium and 199 medium containing peni-

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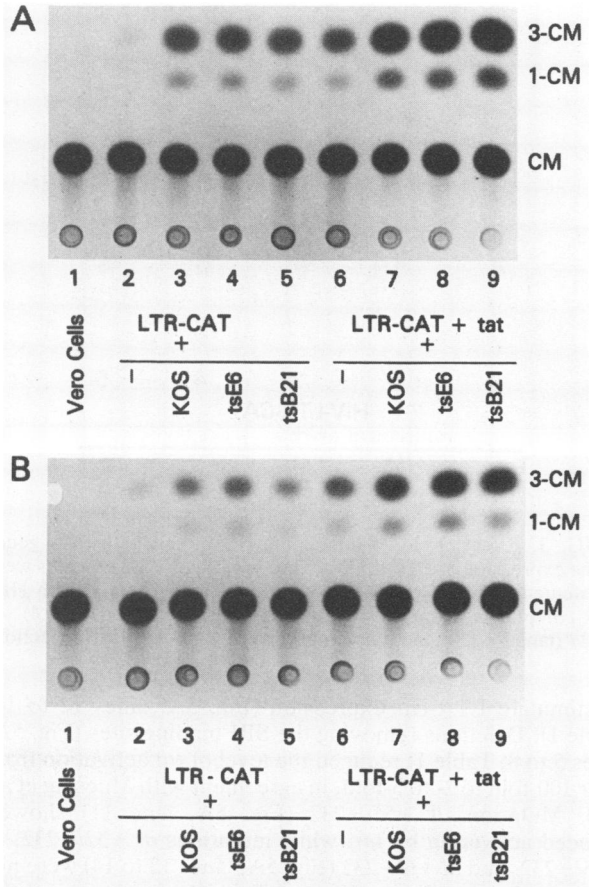

FIG. 1. CAT activity in HSV-infected Vero cells transfected with HIV LTR-*cat*. Cells were either mock transfected (lane 1) or transfected with LTR-*cat* (lanes 2 to 5) or LTR-*cat* plus pAR(tat) (lanes 6 to 9). At 24 h posttransfection, cells were mock infected (lanes 2 and 6) or infected with 5 PFU of wild-type HSV-1 per cell (lanes 3 and 7) or 5 PFU of the temperature-sensitive mutant *ts*E6 per cell (lanes 4 and 8) or *ts*B21 (lanes 5 and 9). Cell extracts were harvested at 20 h postinfection, and CAT activity was assayed. These experiments were carried out at the permissive (34°C; panel A) and nonpermissive (39°C panel B) temperatures. CM, Chloramphenicol.

cillin G (100 U/ml), streptomycin (100 μg/ml), and 10% fetal bovine serum. SW480 cells, a human colon carcinoma cell line (ATCC CCL228), were grown in Dulbecco minimal essential medium as previously described (1). HSV type 1 strain KOS and temperature-sensitive mutants *ts*B21 and *ts*E6 were kindly provided by Priscilla Schaffer.

Plasmids and DNA preparation. The HIV LTR was inserted upstream from the bacterial *cat* gene as previously described (1, 10). An HIV *tat*-expressing plasmid, pAR(tat), was generated from an infectious molecular clone of the virus deleted in the structural and regulatory genes (10). The HIV LTR-*cat* plasmids used in the deletion mutant studies were derived from a parent LTR containing sequences from −525 to +232 relative to the cap site and were generously provided by Dan Capon. These mutants have deletions within the LTR; for example, mutation *dl* −525/−98 lacks sequences from −525 to −98 but contains the LTR sequences from −97 to +232 inserted upstream from the *cat* gene (26). The HSV-containing clones ICP27 and ICP0 are pMC150 and pMC151, respectively, and were gifts of Mark Challberg. The ICP4 clone, pEK1-2, was a gift of Neil DeLuca. All plasmid DNAs were prepared as described by Birnboim and Doly (3) and purified by isopycnic centrifugation in cesium chloride.

Transfections and infections. For CAT assays, cells were grown to 70% confluence in 60-mm-diameter dishes. For transfection, DNA samples were calcium phosphate coprecipitated by the method of Graham and Van der Eb (13). At 4 h after transfection, cells were washed in medium and shocked for 2- to 4-min with 20% dimethyl sulfoxide. The cells were then washed and placed in growth medium. At 24 h posttransfection, the medium was removed and selected cultures were infected with 5 PFU of the appropriate HSV strain per cell. After 1 h, the cells were fed with complete medium and infection was allowed to proceed for an additional 20 h. Transfections involving the infectious clone of HIV were carried out as before but in 25-cm^2 screw-cap tissue culture flasks. For these experiments, the medium was removed at 12-h intervals and stored at −70°C to be assayed later for reverse transcriptase activity.

CAT assays. Cell extracts were prepared as described by Gorman et al. (12). All reaction mixtures were corrected for protein content as determined by the method of Bradford (4). Cell extracts were preheated at 65°C for 5 min before CAT reactions. All reactions were carried out for 30 to 60 min and maintained within the linear range of the assay. Following incubation, reaction mixtures containing 5 μCi of [^{14}C] chloramphenicol (New England Nuclear Corp., Boston, Mass.) were extracted with ethyl acetate, dried, and analyzed by ascending chromatography on silica gel thin-layer chromatography plates in chloroform-methanol (95:5) as previously described. The quantitation of percent conversion was determined by recovering the acetylated and nonacetylated forms of [^{14}C]chloramphenicol from the silica gel plates and counting them in a liquid scintillation counter.

Reverse transcriptase assay. Reverse transcriptase assays with [^{32}P]dTTP were performed by using a modification (R. Willey et al., submitted for publication) of the protocol described by Goff et al. (11). Reaction mixtures (50 μl) containing poly(A) (5 μg/ml) as the template and oligo(dT) (6 μg/ml) as the primer, 50 mM Tris hydrochloride (pH 7.8), 75 mM KCl, 2 mM dithiothreitol, Nonidet P-40 (0.5%), 0.5 mM $MgCl_2$, and [^{32}P]dTTP (Amersham) were placed in individual wells of a 96-well microtiter plate, and 10 μl of culture supernatant was added. The plate was incubated for 3 h at 37°C. Ten microliters of the reaction mixture was spotted onto Whatman DE81 paper, allowed to dry, and washed extensively with 2× SSC (0.3 M sodium chloride, 0.03 M sodium citrate). Autoradiography was performed overnight.

RESULTS

The ability of HSV type 1 to stimulate HIV-LTR-directed gene expression was initially tested in Vero cells, a continuous line of African Green Monkey kidney cells in which HSV replicates efficiently. Cells were transfected with an LTR-*cat* plasmid or cotransfected with both the LTR-*cat* and pAR(tat) plasmids. At 24 h posttransfection, cells were infected with HSV. When cells that had been cotransfected with LTR-*cat* and saturating amounts of *tat* (10) were superinfected with HSV, a further threefold stimulation above that induced by *tat* alone was seen. These results were observed at both 34 and 39°C (Fig. 1A and B, lanes 2, 6, and 7) and at 37°C (data not shown). Infection of cells containing LTR-*cat* by temperature-sensitive mutants of HSV produced comparable levels of *cat* stimulation at both the permissive (34°C) and nonpermissive (39°C) temperatures.

Infection with *ts*E6, which is defective in the immediate-early regulatory protein ICP27, and infection with *ts*B21, which is defective in the immediate-early regulatory protein ICP4, stimulated the HIV promoter to similar degrees (Fig. 1A and B, lanes 4 and 5), and further augmentation was seen in the presence of *tat* (lanes 8 and 9).

To determine which HSV gene products play a role in this stimulatory effect, clones containing individual HSV immediate-early genes were transfected into Vero cells, and Fig. 2 shows their effect on HIV LTR-*cat* expression. Cells were cotransfected with HIV LTR-*cat* and either pAR(tat), ICP0, ICP4, or ICP27 or with a combination of pAR(tat) and the individual HSV immediate-early genes. ICP0, ICP4, or ICP27 had no effect on *cat* expression in the absence of *tat*. In the presence of pAR(tat), ICP4 and ICP0 produced a three- to fourfold stimulatory effect on LTR-*cat* expression above that seen with pAR(tat) alone, while ICP27 had no effect. These results differ from those seen in SW480 cells, in which ICP4 showed a marked stimulatory effect on CAT activity, while ICP0 and ICP27 produced little and r effect, respectively (Fig. 3).

To identify the HIV LTR sequences which respond to HSV activation, a series of LTR-*cat* deletion mutations from the 5′ and 3′ ends of pLTR (−525/+232 LTR sequences) (26) were tested for their ability to be stimulated by HSV infection. Figure 4 shows the entire sequence of the HIV LTR and indicates many of its known regulatory elements (26, 34, 35, 37–39). The LTR sequences deleted in each construct are noted by black bars. Table 1 displays the basal levels of expression of the pLTR-*cat* deletion constructs in Vero cells. Plasmids with sequences deleted between −525/−77 and −525/−30, which remove the SP1 binding sites, had reduced basal levels of expression. Figure 5A (and Table 1) demonstrates the effect of *tat* cotransfection on these mutations. Two areas of the LTR seem to be necessary for optimal activation by *tat*. Deletions within the TAR region (−17/+80) had a profound effect on the ability of *tat*

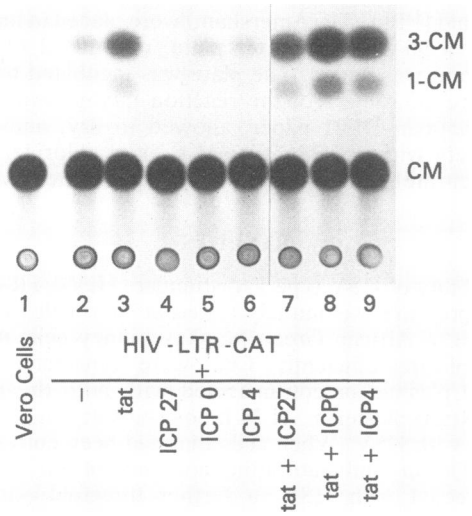

FIG. 2. Effect of clones containing HSV immediate-early genes on the expression of HIV LTR-*cat*. Vero cells were mock transfected (lane 1) or cotransfected with HIV LTR-*cat* alone (lane 2), HIV LTR-*cat* plus pAR(tat) (lane 3), ICP27 (lane 4), ICP0 (lane 5), ICP4 (lane 6), pAR(tat) plus ICP27 (lane 7), pAR(tat) plus ICP0 (lane 8), or pAR(tat) plus ICP4 (lane 9). CM, Chloramphenicol.

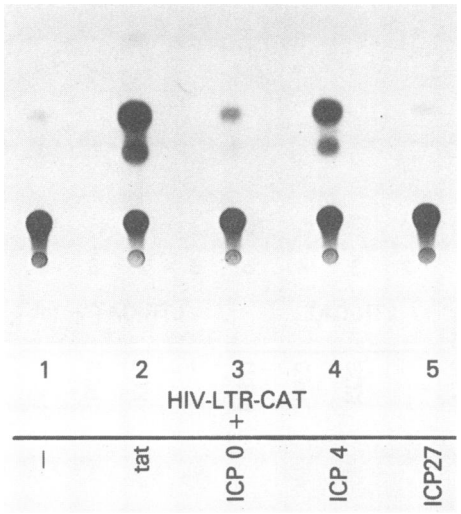

FIG. 3. Effect of clones containing HSV immediate-early genes on the expression of HIV LTR-*cat* in SW480 cells. SW480 cells were transfected with HIV LTR-*cat* (lane 1) or cotransfected with HIV LTR-*cat* plus pAR(tat) (lane 2), ICP0 (lane 3), ICP4 (lane 4), or ICP27 (lane 5). CAT activity was assayed at 48 h posttransfection.

to stimulate LTR-*cat* expression (Fig. 5A, lanes 12 to 14; Table 1). Deletions removing the SP1 binding sites (Fig. 5A, lanes 5 to 8; Table 1) reduced the level of *tat* activation from over 100-fold to 2- to 5-fold, in agreement with Muesing et al. (26). Mutation *dl* +55/+232 (Fig. 5A, lane 11) showed reduced activation by *tat*, while mutations *dl* +32/+232, *dl* +23/+232, and *dl* +6/+232 (Fig. 5A, lanes 12 to 14) were not activated by *tat*. Figure 5B (and Table 1) demonstrates the effect of HSV infection on cells transfected with pLTR-*cat* and the series of deletion mutations. Lanes 10 to 14 show that, when infected with HSV, cells transfected by plasmids with deletions in which the entire TAR region was removed showed levels of CAT activity similar to those of undeleted LTR-*cat*. There was an 80 to 100% decrease in CAT activity in cells transfected with plasmids containing deletions that extend into the SP1 binding sites and then infected with HSV (Fig. 5B, lanes 6 to 8). All other mutations showed greater than 100-fold activation by HSV infection. Table 1 shows the percent acetylation of chloramphenicol from Fig. 5A and B.

The experiments with HIV LTR-*cat* constructions demonstrated the stimulatory effects of HSV on HIV LTR activity and allowed identification of the HSV genes and HIV regulatory sequences involved. To determine whether the effects of HSV activation on the LTR-*cat* constructs promote the replication of HIV, an infectious clone of HIV was cotransfected into HeLa cells with the individual immediate-early genes of HSV. Figure 6 shows the effect on HIV replication of clones containing the individual HSV immediate-early gene products ICP0, ICP4, and ICP27 and an HSV-1 DNA clone (pEcoC) that contains all three immediate-early genes (*Eco*RI-C). Reverse transcriptase activity in the supernatants of HeLa cell cultures was assayed. Beginning at 24 h posttransfection, reverse transcriptase activity could be detected easily, whereas mock-infected cultures had no activity (Fig. 6, lanes 1 and 2). Cotransfection of cells with HSV, pEcoC, or the clones containing ICP4 or ICP0 had a five- to eightfold stimulatory effect on reverse transcriptase expression (lanes 3, 5, and 6), while ICP27 had no effect. These data are consistent with those of Fig. 2 and 3, showing the stimulatory effects of both ICP4 and ICP0.

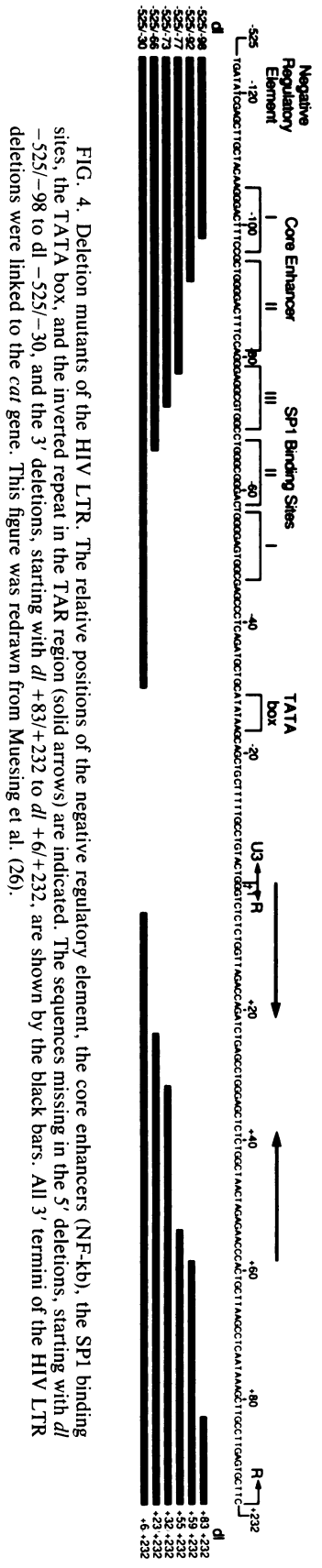

FIG. 4. Deletion mutants of the HIV LTR. The relative positions of the negative regulatory element, the core enhancers (NF-kb), the SP1 binding sites, the TATA box, and the inverted repeat in the TAR region (solid arrows) are indicated. The sequences missing in the 5' deletions, starting with *dl* −525/−98 to dl −525/−30, and the 3' deletions, starting with *dl* +83/+232 to *dl* +6/+232, are shown by the black bars. All 3' termini of the HIV LTR deletions were linked to the *cat* gene. This figure was redrawn from Muesing et al. (26).

TABLE 1. Effect of *tat* or HSV infection on expression of HIV LTR or HIV LTR deletion mutants

Plasmid	% Acetylation		
	Plasmid	*tat*	HSV
LTR-*cat*	0.12	8.5	16.5
dl −525/−98	0.32	31.3	24.9
dl −525/−92	0.64	97.9	47.5
dl −525/−77	0.093	1.16	7.3
dl −525/−73	0.094	0.46	2.7
dl −525/−66	0.086	0.16	0.37
dl −525/−30	0.13	0.62	3.12
dl +83/+232	0.52	70.5	47.3
dl −59/+232	2.14	33.5	40.8
dl +55/+232	0.53	4.8	39.4
dl +32/+232	0.89	1.5	37.9
dl +23/+232	0.50	0.95	14.5
dl +6/+232	0.31	0.22	25.7

DISCUSSION

We have shown that HSV type 1 and its immediate-early genes ICP0 and ICP4 greatly stimulate the expression of HIV LTR-directed viral gene expression, as measured by the production of CAT. The stimulatory effects of HSV and some of its immediate-early genes may have implications for HIV-induced disease, since increased reverse transcriptase activity, which is indicative of virion production, was detected when an infectious clone of HIV was cotransfected with both ICP0 and ICP4. Three distinct genes of HSV have been shown to code for *trans*-activating factors: ICP0, which can *trans*-activate most genes introduced into cells in a nonspecific manner; ICP4, a gene required for specific activation of HSV genes of the β and γ classes; and α-*trans*-inducing factor, a structural component of the virion which activates immediate-early genes of HSV which share the common sequence TAATGARATTC (R, purine). Variations on this sequence have been found in other nonherpesvirus genes, including the noncoding strand of the Moloney murine sarcoma virus LTR (33).

The activation of the HIV LTR by HSV is dependent on the type of cell used in the experiment, in that ICP4 and ICP0 were active in Vero cells only in the presence of *tat*, while ICP4 and, to a much lesser extent, ICP0 activated the HIV LTR in SW480 cells in the absence of *tat*. Studies with deletion mutations showed that two regions within the LTR are important for *trans*-activation by *tat* and HSV. The TAR region (bases −17 to +80) is required for *tat* activation, but deletions in this area had no effect on the stimulatory effects of HSV in Vero or SW480 cells (data not shown) or of ICP4 in SW480 cells (data not shown). Deletions that remove upstream sequences that include the SP1 binding sites (base pairs −78 to −47) markedly reduce the effects of HSV, ICP4 alone, and *tat* on LTR expression.

The ability of one virus to augment the expression of another virus or rescue it from a latent state has been previously described (5, 31). It has been shown that adenovirus and herpes pseudorabies virus immediate-early genes can activate expression of human T-lymphotropic virus type 1 and II LTRs and also that HSV and adenovirus can rescue latent adeno-associated virus and provide helper functions for its replication (5, 31). In addition to HSV, we have previously shown that varicella-zoster virus, JC virus, BK virus, and papillomavirus genes transcriptionally activate the HIV LTR, in that increased levels of CAT and

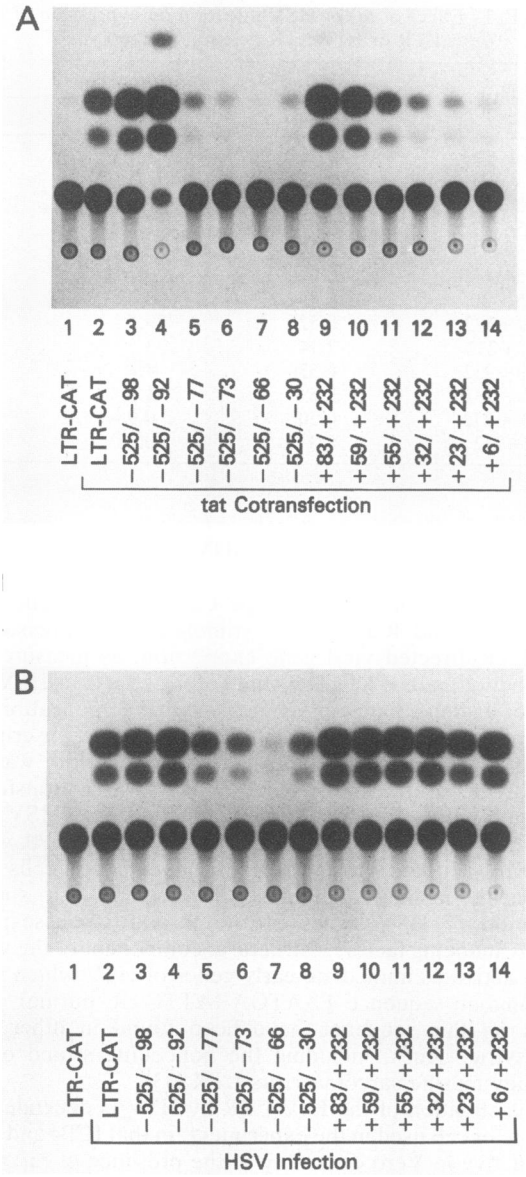

FIG. 5. The effect of pAR(tat) cotransfection or HSV infection on the expression of *cat* by HIV LTR-*cat* deletion mutants. Vero cells were transfected with LTR-*cat* (lanes 1 and 2) or the series of 5′ and 3′ LTR-*cat* deletion mutants (lanes 3 to 14). Panel A shows the results of CAT assays when pAR(tat) was cotransfected with the LTR-*cat* constructs, and panel B shows the results of HSV infection (5 PFU per cell) on cells previously (24 h) transfected with the HIV LTR-*cat* constructs.

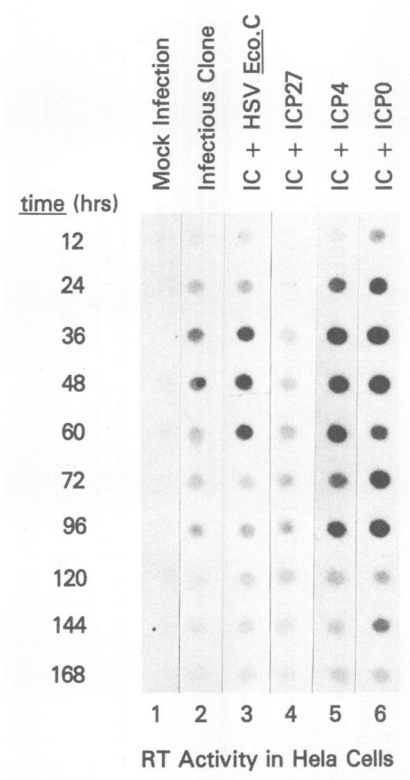

FIG. 6. The effect of clones containing HSV immediate-early genes on HIV replication. HeLa cells were mock transfected (lane 1), transfected with an infectious clone (IC) of HIV (lane 2), or cotransfected with the IC plus HSV pEco-C (lane 3), the IC plus ICP27 (lane 4), the IC plus ICP4 (lane 5), or the IC plus ICP0 (lane 6). Supernatant fluid was replaced at the indicated times, and the reverse transcriptase activity of a sample was assayed as described in Materials and Methods. RT, Reverse transcriptase.

LTR-*cat* RNA were detected. The ability of herpes simplex virus to activate an HIV LTR integrated into Vero cells has also been reported (25).

The data presented here indicate that individual HSV gene products can participate in LTR activation and the mechanism of activation may vary in different cell lines. The observation that ICP4 is able to induce activation of HIV LTR-*cat* in SW480 but not in Vero cells suggests that SW480 cells already contain a cellular protein(s) that is otherwise not present in Vero cells. Such a cellular protein would be required for activation of the LTR by the cloned immediate-early genes of HSV. The fact that HIV-LTR activation by HSV infection occurs in Vero cells in the absence of *tat* implies that HSV codes for, or introduces into the cell, a protein that induces this putative cellular factor. Productive replication of HSV is not necessary to induce this factor, since *ts* mutants still activate the HIV LTR at the nonpermissive temperature. Under these conditions, even though the virus does not replicate, all other immediate-early gene products are expressed and the *trans*-activating virion component α-*trans*-inducing factor is introduced into the cell. This may be responsible for the induction of this putative cellular protein.

The observation that transfection of a *tat*-negative proviral DNA results in some progeny virus production in SW480 cells, but much less in Vero cells, is also consistent with the hypothesis that SW480 cells contain a cellular factor(s) not found in Vero cells which affects LTR-directed viral gene synthesis (H.E.G., unpublished data). It has been postulated that HSV ICP4 *trans*-activates HSV early genes by interacting with a cellular protein which in turn binds to sequences within HSV early gene promoters (21). Infection of cells with HSV may induce a number of cellular transcription factors that are also necessary for HIV LTR activation. SP1 and NF-kB are two such transcription-activating proteins that are known to bind to the HIV LTR (18, 27). Three SP1 binding sites have been mapped to the area between −46 and −78, and deletions encompassing this region fail to demonstrate activation by HSV. Therefore, SP1 may be in part responsible for the HSV effect. Two NF-kB binding sites have been mapped to −80 to −92 and −96 to −106, both in

the region that has been called the core enhancer. Deletions removing the 5'-most binding site are still responsive (*dl* −525/−92) to HSV activation, while the deletion that removes both of these binding sites, as well as the third SP1 site, has less than 10% of the activity of the intact LTR. Deletions that extend further in the 3' direction and remove SP1 binding sites II and III are not activatable with HSV. It is of interest that *dl* −525/−30, which removes all upstream regulatory sequences and contains only a TATA box, has some activity following infection with HSV. This observation is consistent with that of Johnson and Everett, who showed that control of certain HSV late transcription units requires only a TATA box and cap site region (17). Taken together, these data imply that HSV infection induces cellular factors that affect HIV replication and may be involved in the activation of proviral DNA.

In vitro studies of HIV indicate that viral replication is, to some extent, dependent on the activation of infected host cells. For example, active replication of HIV in $CD4^+$ lymphocytes requires stimulation of those cells (14, 42). Clinically, both the variable incubation period of acquired immunodeficiency syndrome and the small number of HIV-infected cells detected in the peripheral blood of seropositive individuals (15) suggest that viral replication may occur at only very low levels early after infection. HIV has also been detected in other cell types, including monocytes (20). Recently, cellular colocalization of human cytomegalovirus and HIV has been reported to occur in the white matter of the brain of an acquired immunodeficiency syndrome patient (J. A. Nelson, C. Wiley, C. Reynolds, and M. B. A. Oldstone, Abstr. 12th Int. Herpesvirus Workshop, abstr. no. 341, 1987). Thus, a variety of factors participate in the activation of HIV, ones with a profound impact on the progression of HIV infection to full-blown acquired immunodeficiency syndrome. The ability of HSV to activate HIV provides a model of how concomitant infections with other viruses could enhance HIV replication.

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LITERATURE CITED

1. **Adachi, A., H. E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, and M. A. Martin.** 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. J. Virol. **59**:284–291.
2. **Barré-Sinoussi, F., J. C. Cherman, R. Rey, M. T. Nugeybe, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rosenbaum, and L. Montagnier.** 1983. Isolation of a T-lymphotrophic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science **220**: 868–871.
3. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
4. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–264.
5. **Chen, I. S. Y., A. J. Cann, N. P. Shah, and R. B. Gaynor.** 1985. Functional relation between HTLV-IIx and adenovirus E1A proteins in transcriptional activation. Science **230**:570–573.
6. **Cullen, B. R.** 1986. *trans*-Activation of human immunodeficiency virus occurs via a bimodal mechanism. Cell **46**:973–982.
7. **Dynan, W. S., and R. Tjian.** 1985. Control of eukaryotic messenger RNA synthesis by sequence-specific DNA-binding proteins. Nature (London) **316**:774–778.
8. **Everett, R. D., and M. Dunlop.** 1984. *trans* activation of plasmid-borne promoters by adenovirus and several herpes group viruses. Nucleic Acids Res. **12**:5969–5977.
9. **Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, P. Foster, and P. D. Markham.** 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science **224**: 500–503.
10. **Gendelman, H. E., W. Phelps, L. Feigenbaum, J. M. Ostrove, A. Adachi, P. M. Howley, G. Khoury, H. S. Ginsberg, and M. A. Martin.** 1986. *trans*-Activation of the human immunodeficiency virus long terminal repeat sequence by DNA viruses. Proc. Natl. Acad. Sci. USA **83**:9759–9763.
11. **Goff, S., P. Traktman, and D. Baltimore.** 1981. Isolation and properties of Moloney murine leukemia virus mutants: use of a rapid assay for release of virion reverse transcriptase. J. Virol. **38**:239–248.
12. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
13. **Graham, F. L., and A. J. Van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–467.
14. **Harada, S., Y. Koyanag, H. Nakashima, N. Kobayashi, and N. Yamamoto.** 1986. Tumor promoter, TPA, enhances replication of HTLV-III/LAV. Virology **154**:249–258.
15. **Harper, M. E., L. M. Marselle, R. C. Gallo, and F. Wong-Staal.** 1986. Detection of lymphocytes expressing human T-lymphotropic virus type III in lymph nodes and peripheral blood from infected individuals by *in situ* hybridization. Proc. Natl. Acad. Sci. USA **83**:772–776.
16. **Honess, R. W., and B. Roizman.** 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. **14**:8–19.
17. **Johnson, P. A., and R. D. Everett.** 1986. The control of herpes simplex virus type-1 late gene transcription: a 'TATA-box'/cap site region is sufficient for fully efficient regulated activity. Nucleic Acids Res. **14**:8247–8264.
18. **Jones, K. A., J. Kadonaga, P. A. Luciw, and R. Tjian.** 1986. Activation of the AIDS retrovirus promoter by the cellular transcription factor, Sp1. Science **232**:755–759.
19. **Jones, K. A., and R. Tjian.** 1985. Sp1 binds to promoter sequences and activates herpes simplex virus 'immediate-early' gene transcription *in vitro*. Nature (London) **317**:179–182.
20. **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. Fauci.** 1986. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. Science **233**:1089–1093.
21. **Kristie, T. M., and B. Roizman.** 1987. Host cell proteins bind to the *cis*-acting site required for virion-mediated induction of herpes simplex virus 1 α genes. Proc. Natl. Acad. Sci. USA **84**:71–75.
22. **Levy, J. A., A. D. Hoffman, S. M. Kramer, J. A. Lanois, J. M. Shimabukuro, and L. S. Oskiro.** 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science **225**:840–842.
23. **McKnight, S., and R. Tjian.** 1986. Transcriptional selectivity of viral genes in mammalian cells. Cell **46**:795–805.
24. **Melbye, M., R. J. Biggar, P. Ebbesen, C. Neuland, J. J. Goedert, V. Faber, I. Lorenzen, J. P. Skinh, R. C. Gallo, and W. A. Blattner.** 1986. Long-term seropositivity for HTLV-III in homosexual men without the acquired immunodeficiency syndrome: development of immunologic and clinical abnormalities: a longitudinal study. Ann. Intern. Med. **104**:496–500.
25. **Mosca, J. D., D. P. Bednarik, N. B. K. Raj, C. A. Rosen, J. G. Sodroski, W. A. Haseltine, and P. M. Pitha.** 1987. Herpes simplex virus type-1 can reactivate transcription of latent human immunodeficiency virus. Nature (London) **325**:67–70.
26. **Muesing, M. A., C. J. Smith, and D. H. Capon.** 1987. Regulation of mRNA accumulation by a human immunodeficiency virus

trans-activator protein. Cell **48**:691–701.
27. **Nabel, G., and D. Baltimore.** 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature (London) **326**:711–713.
28. **Nahmias, A. J., S. Kibrick, and R. C. Rosan.** 1964. Viral leukocyte interrelationships. I. Multiplication of a DNA virus—herpes simplex—in human leukocyte cultures. J. Immunol. **93**:69–74.
29. **O'Hare, P., and G. S. Hayward.** 1985. Evidence for a direct role for both the 175,000- and 110,000-molecular-weight immediate-early proteins of herpes simplex virus in the transactivation of delayed-early promoters. J. Virol. **53**:751–760.
30. **O'Hare, P., and G. S. Hayward.** 1985. Three *trans*-acting regulatory proteins of herpes simplex virus modulate immediate-early gene expression in a pathway involving positive and negative feedback regulation. J. Virol. **56**:723–733.
31. **Ostrove, J. M., and K. I. Berns.** 1980. Adenovirus early region 1b gene function required for rescue of latent adeno-associated virus. Virology **104**:502–505.
32. **Rinaldo, C. R., Jr., B. S. Richter, P. H. Black, R. Callery, L. Chess, and M. S. Hirsch.** 1978. Replication of herpes simplex virus and cytomegalovirus in human leukocytes. J. Immunol. **120**:130–136.
33. **Roizman, B., T. Kristie, W. Batterson, and S. Mackem.** 1984. The regulation of α genes of herpes simplex virus 1, p. 227–238. *In* H. S. Ginsberg and H. S. Vogel (ed.), Transfer and expression of eukaryotic genes. Academic Press, Inc., New York.
34. **Rosen, C., J. G. Sodroski, W. Goh, A. Dayton, J. Lippke, and W. A. Haseltine.** 1986. Post-transcriptional regulation accounts for the *trans*-activation of the human T-lymphotrophic virus type III. Nature (London) **319**:555–559.
35. **Rosen, C., J. G. Sodroski, and W. A. Haseltine.** 1985. Location of *cis*-acting regulatory sequences in the human T-lymphotrophic virus type III (HTLV-III/LAV) long terminal repeat. Cell **41**:813–823.
36. **Sacks, W. R., C. C. Greene, D. P. Aschman, and P. A. Schaffer.** 1985. Herpes simplex virus type 1 ICP27 is an essential regulatory protein. J. Virol. **55**:796–805.
37. **Sodroski, J. G., W. C. Goh, C. Rosen, K. Campbell, and W. A. Haseltine.** 1986. Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. Nature (London) **322**:470–474.
38. **Sodroski, J. G., W. C. Goh, C. Rosen, A. I. Dayton, E. Terwilliger, and W. A. Haseltine.** 1986. A second post-transcriptional *trans*-activator gene required for HTLV-III replication. Nature (London) **321**:412–417.
39. **Sodroski, J. G., C. A. Rosen, and W. A. Haseltine.** 1984. *trans*-Acting transcriptional activation of the human T lymphotropic virus long terminal repeat in infected cells. Science **225**:381–384.
40. **Sodroski, J. G., C. A. Rosen, F. Wong-Staal, S. Z. Salahuddin, M. Popovic, S. Arya, R. C. Gallo, and W. A. Haseltine.** 1985. *trans*-Acting transcriptional regulation of human T-cell leukemia virus type III long terminal repeat. Science **227**:171–173.
41. **Wright, C. M., B. K. Felber, H. Paskalis, and G. N. Pavlakis.** 1986. Expression and characterization of the *trans*-activator of HTLV-III/LAV virus. Science **234**:988–992.
42. **Zagury, D., J. Bernard, R. Leonard, R. Cheynier, M. Feldman, P. S. Sarin, and R. C. Gallo.** 1986. Long-term cultures of HTLV-III-infected T cells: a model of cytopathology of T-cell depletion in AIDS. Science **231**:850–853.