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Expression of the Herpes Simplex Virus 1 α Transinducing Factor (VP16) Does Not Induce Reactivation of Latent Virus or Prevent the Establishment of Latency in Mice

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A feature of the cascade regulation of herpes simplex virus 1 gene expression in productive infection is that the first genes to be expressed, the α genes, are transactivated by a structural component of the virion designated as the α transinducing factor (αTIF). In this study, we have tested the hypothesis that latent infection of sensory neurons results from the failure of αTIF, a tegument protein, to be transported from the nerve endings to the nucleus of the sensory neuron. Two viruses were constructed. The first recombinant virus (R6003) contained a second copy of the αTIF gene placed under the control of a metallothionein promoter. The second recombinant virus (R6004) is identical to R6003 except for the presence of a stop codon inserted at amino acid 70 of the second αTIF gene. The metallothionein promoter inserted into the viral genome was shown to be expressed, and αTIF mRNA was detected by in situ hybridization of sections of trigeminal ganglia of mice infected with R6003, both untreated and those given cadmium injections. In all experiments, there were no significant differences in the recovery of latent virus from mice infected with R6003 or R6004, whether injected with cadmium or not. Cadmium administration at the time of infection and at intervals thereafter did not preclude establishment of latency. In another series of experiments, transgenic mice expressing the metallothionein-driven αTIF did not differ from nontransgenic siblings with respect to the incidence of latent virus in trigeminal ganglia. We conclude that the absence of αTIF cannot alone account for the establishment of latency.

The experiments described in this report test a hypothesis described earlier (33): that herpes simplex virus (HSV) latency results from the absence of a vital viral transactivating factor.

In HSV-1-infected cells, viral gene expression is regulated in a cascade fashion (14, 15). The expression of α genes, the first set of genes to be expressed, is transactivated by a viral structural protein (3, 32). The transactivator has been designated as the α transinducing factor (αTIF) according to its function (30) or VP16 according to the nomenclature assigned to virion proteins (36), and it is present in 500 to 1,000 copies per virion in the structure designated as the tegument located between the envelope and the capsid (13). Upon entry of the virus into the cell, the capsid migrates to the nuclear pore and the DNA is released into the nucleus, where it circularizes (2, 31). A *cis*-acting site found upstream of all α gene promoters (20, 24) is bound by a host protein designated variously as αH1, OTF-1, Oct-1, or NF-III (9, 21, 27, 28). αTIF binds to the Oct-1–DNA complex (21, 22). Experiments showing that αTIF transactivated a viral gene resident in the host genome under conditions of infection in which the viral DNA was not released from the capsid indicated that αTIF makes its way to the nucleus independently of the viral DNA (3). As noted previously (33), the puzzling features of the regulation of HSV gene expression are (i) the use of a transactivator rather than of enhancer elements for expression of α genes, (ii) the physical separation of the DNA and transactivator in different compartments of the virion, and (iii) the use of a cellular transactivator, i.e., Oct-1, as the linker between αTIF and its *cis*-acting site.

HSV can establish latency in sensory neurons both in humans and in experimental animal systems. In neurons harboring latent virus, the only viral transcript detected to date (the latency-associated transcript [LAT]) accumulates in nuclei (37) and has not been shown to encode a protein. Mutants which do not produce the LAT are not defective with respect to the establishment of latency (17). Attempts to identify the gene(s) required for the establishment of the latent state have not been successful. While reactivation from the latent state requires competence to multiply (6, 16, 18), virtually any deletion mutant administered to the mouse at appropriate sites and in appropriate amounts can establish latency (18, 23, 26, 34, 38). These results seem to negate the hypothesis that latency requires the expression of a viral gene. Inasmuch as neuronal cells in culture are permissive for lytic infection by HSV, and latent virus can be reactivated from sensory neurons in vivo, the latently infected sensory neuron must be viewed as only transiently nonpermissive.

One hypothesis that could explain the nonpermissiveness of the neuronal cells in situ is that replication cannot take place because the α genes are not transactivated (33). For example, the distance which separates the plasma membrane of a cell in a mucous membrane from its nucleus, the site of activation of α genes by αTIF, is measured in micrometers. The distance between the peripheral endings of a sensory

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neuron ennervating a mucous membrane and the neuronal nucleus is measured in centimeters. It is conceivable that latency results from a failure of αTIF to be transported to the neuronal nucleus. To test this hypothesis, we constructed two viruses. The first virus contains a second copy of αTIF placed under a metallothionein I (MT-I) promoter (MT-αTIF). The second virus is identical to the first except that we inserted a stop codon into the MT-αTIF gene after sequences encoding the first 70 amino acids.

Although we have shown that in sensory neurons infected with recombinant viruses the MT-I promoter is functional and that the αTIF gene is expressed, we have detected no evidence that αTIF terminates or precludes the establishment of latency. Similarly, we find that transgenic mice expressing the same MT-αTIF gene are not resistant to the establishment of latency after inoculation of HSV-1.

MATERIALS AND METHODS

Cells. Vero and rabbit skin cells were obtained from the American Type Culture Collection. The 143TK$^-$ cells were a gift of C. Croce. HAT medium has been described elsewhere (5).

Viruses. HSV-1 strain F is the prototype HSV-1 strain used in this laboratory. HSV-1(F)Δ305 was derived from HSV-1(F) and lacks the *Bgl*II-*Sac*I fragment of the thymidine kinase (*tk*) gene; its construction has been described elsewhere (32). R314 was derived from HSV-1(F) by insertion of the 1.8-kbp *Bam*HI Z fragment into the *Bgl*II restriction endonuclease site of the *Bam*HI Q fragment encoding the *tk* gene. The *Bam*HI Z fragment was inserted in an orientation such that the α4 gene promoter, containing all upstream sequences from approximately −1800 to +33, is juxtaposed to and drives the expression of the *tk* gene containing all sequences downstream from +51 (32).

Infections of mice. Five-week-old male BALB/c AnN mice (Charles River) were infected in both eyes under sodium pentobarbital anesthesia. The corneas were scarified with a 30-gauge hypodermic needle, and the virus was placed on the eye in 10 μl of medium 199V (mixture 199 supplemented with 1% newborn calf serum).

Cadmium sulfate injections. $CdSO_4$ was injected subcutaneously into footpads as a 2-mg/ml solution in sterile saline. Each mouse received 100 μg in 50 μl. This dose of cadmium has been demonstrated to cause induction of the MT-I promoter in vivo (29).

Assays of infectious and latent virus in ganglia. Mice were sacrificed under sodium pentobarbital anesthesia, and trigeminal ganglia were removed and placed in 1 ml of DME-5 medium (Dulbecco's modified Eagle's supplemented with 5% newborn calf serum, 125 U of penicillin per ml, 0.125 mg of streptomycin sulfate per ml, and 25 U of mycostatin per ml). In assays for latent virus, the ganglia were incubated in this medium for 5 days in 5% CO_2 at 37°C, homogenized, and plated on Vero cell monolayers (one 25-cm^2 culture per ganglion). After 1 h at 37°C to allow virus to attach to cells, the homogenate was removed and the cells were incubated at 37°C in 5 ml of DME-5 medium. Cultures were observed daily for at least 6 days and were scored as positive if any plaques developed during that period. For infectious virus assays, ganglia were homogenized within 2 h of removal from the animals and plated on Vero cell monolayers in the same manner as those assayed for latent virus. Cultures were observed daily for at least 6 days.

Assays of infectious virus in eyes. Eyes were bathed in 10 μl of sterile saline solution (0.15 M NaCl), which was removed from the eye and immediately added to 1 ml of DME-5 medium. The medium plus saline was plated on Vero cell monolayers as described above and observed daily for at least 6 days for plaque formation.

RNA probes for in situ hybridizations. The ^{35}S-labeled RNA probes were prepared from linearized plasmid templates by transcription with the SP6 or T7 polymerase, provided with the labeling kit (SP6/T7 RNA labeling kit; Boehringer, Mannheim, Germany). The labeled nucleotide was [^{35}S]UTP (1,000 Ci/mmol; Amersham, Amersham, United Kingdom). The radiolabeled RNA probes were purified by phenol extraction, chromatography through Quick-Spin G-50 columns (Boehringer), and ethanol precipitation. The sizes of the transcripts were determined by electrophoresis on formaldehyde-agarose gels. The probe used to detect αTIF mRNA was transcribed by SP6 polymerase from a plasmid containing the αTIF gene in the pGEM3Z (Promega) vector (pRB3623; Fig. 1, line 6), linearized by *Mlu*I. The product of the transcription was 625 nucleotides long with 475-nucleotide complementarity to αTIF mRNA. The negative control was an RNA transcribed in the opposite direction from the same plasmid linearized at the vector *Hin*dIII site, homologous to αTIF mRNA. The probe for LAT was a 2-kb RNA transcribed from a plasmid containing the *Hpa*I-*Bam*HI fragment of the junction region (Fig. 1), linearized at the *Sal*I site of the fragment and containing RNA sequences from the entire fragment, complementary to LAT. The probes were freeze-dried before solubilization in hybridization buffer.

In situ hybridization. Sections of paraffin-embedded trigeminal ganglia were cut at 5-μm thickness and mounted on organosiliconized slides (25). The sections were pretreated as described by Gordon et al. (10). After deparaffination, the sections were treated with 0.2 N HCl and proteinase K (1 μg/ml; 15 min at 37°C) and acetylated. Prior to hybridization, the sections were subjected to prehybridization at 45°C for 2 to 4 h. The prehybridization solution consisted of 50% formamide, 2× SSC (SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 1× Denhardt's solution, and 300 μg of denatured salmon sperm DNA per ml. The probes were solubilized in hybridization solution at a concentration of 0.5 μg/ml. The hybridization solution contained 50% formamide, 10% dextran sulfate, 0.3 M NaCl, 5 mM Tris (pH 7.4), 1 mM EDTA, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 1 mg of bovine serum albumin per ml, 5 mM dithiothreitol, and 0.5 mg of mouse brain nucleic acids per ml. Twenty microliters of probe solution was applied to each section, and the sections were hybridized for 18 h at 45°C under a sealed cap in a humidified incubator.

After hybridization with RNA probes, the sections were digested with RNase A (60 μg/ml) and RNase T_1 (10 U/ml) for 40 min at 37°C, washed for 72 h in a 2-liter volume of 45% formamide–2× SSC–10 mM Tris (pH 7.4)–1 mM EDTA with one buffer change, subjected to autoradiography for 7 days using NTB-3 emulsion (Eastman Kodak), developed, and stained with hematoxylin-eosin.

RESULTS

To assess the role of αTIF in latent infection, viruses were constructed which expressed either a fully functional αTIF gene or a translationally truncated nonfunctional αTIF gene under the control of a host promoter expressed in sensory neurons. These genes were present in the viral genome in addition to the natural αTIF gene specified by HSV-1. Mice were infected with the recombinant viruses, and the effects

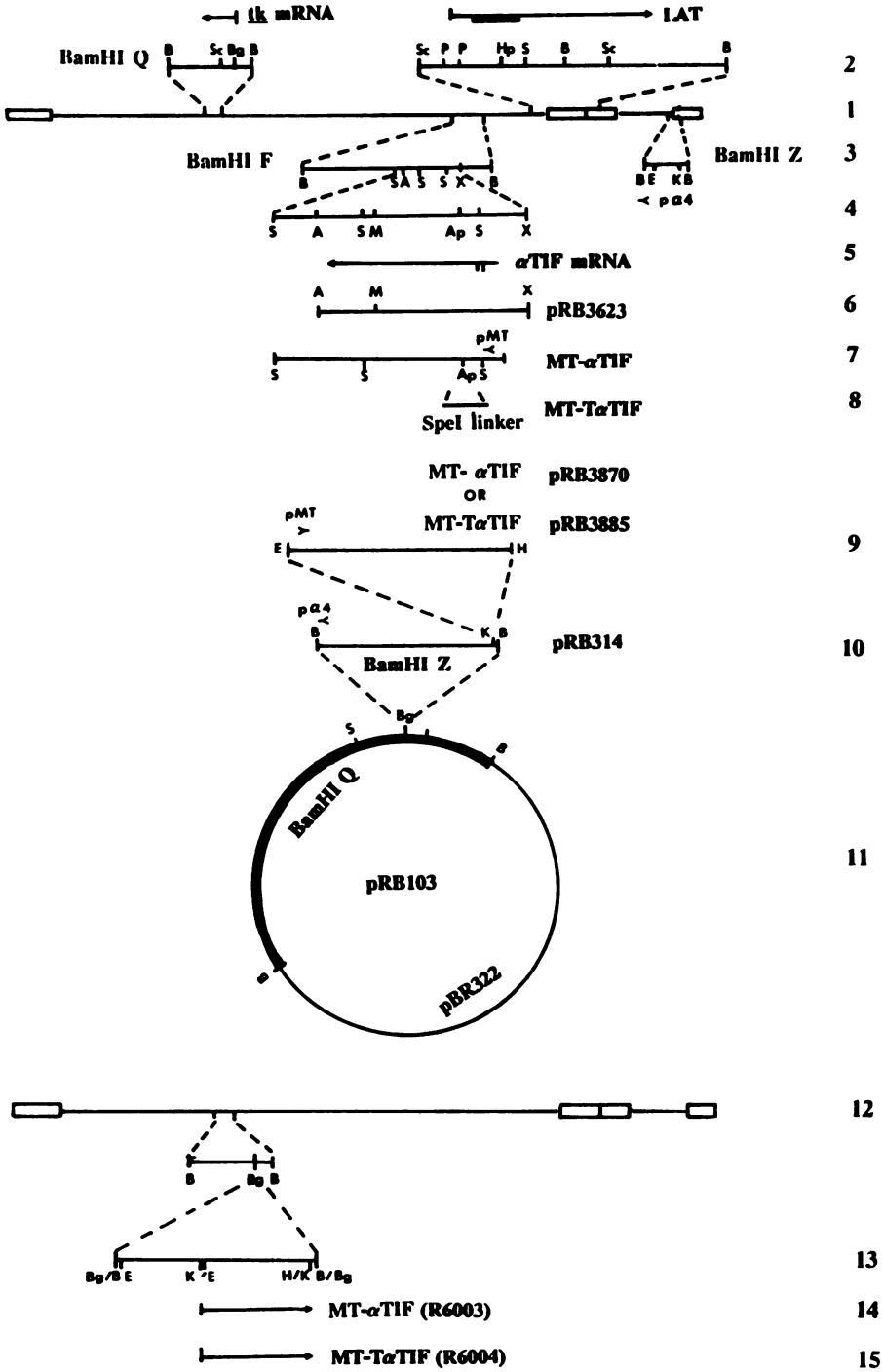

FIG. 1. Sequence arrangements and genomic locations of viruses and plasmids used. Line 1, genomic arrangement of HSV-1(F). Open boxes indicate the locations of the terminal and internal inverted repeat sequences. Line 2, expansion of the *Bam*HI Q fragment and the L-S junction region. Locations of the *tk* gene mRNA and the LAT are shown above the line. The heavy line indicates the portion of LAT that accumulates in latently infected nuclei (7). Line 3, expansions of the *Bam*HI F and Z fragments. The location of the α4 gene promoter (pα4) is indicated by the arrow under the expansion of *Bam*HI-Z. Line 4, expansion of the region of *Bam*HI-F that encodes the αTIF gene. Line 5, location of the αTIF mRNA. Line 6, sequences included in pRB3623. Line 7, construction of the MT-αTIF gene. The MT-I promoter (pMT) from −147 to +57 was fused to the αTIF gene from a *Sal*I site 7 bp upstream of the second ATG to a *Sal*I site downstream of the polyadenylation site. Line 8, insertion of *Spe*I linker, containing stop codons in all six open reading frames, into the unique *Apa*I site of the MT-αTIF gene, creating the MT-TαTIF gene. Line 9, insertion of the MT-αTIF or MT-TαTIF gene into the *Kpn*I site of pRB314, constructing pRB3870 (MT-αTIF-αTK) or pRB3885 (MT-TαTIF-αTK). Both plasmids contain the αTIF genes inserted in the same orientation. The location of the MT-I promoter (pMT) in each case is indicated by the arrow. Line 10, insertion of *Bam*HI-Z into the unique *Bgl*II site in the *tk* gene in *Bam*HI-Q of pRB103, creating a chimeric α4-regulated *tk* gene in pRB314. The location of the α4 promoter (pα4) is indicated by the arrow. Line 11, the *Bam*HI Q fragment inserted into the *Bam*HI site of pBR322. Line 12, genomic arrangement of HSV-1(F). Open boxes indicate the locations of the terminal and internal inverted repeat sequences. Line 13, expansion of the sequence arrangement of the MT-αTIF or MT-TαTIF insertion in the viral genome. Line 14, location of the MT-αTIF gene in recombinant virus R6003. Line 15, location of the MT-TαTIF gene in recombinant virus R6004. Restriction enzymes: A, *Asu*II; Ap, *Apa*I; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; Hp, *Hpa*I; K, *Kpn*I; M, *Mlu*I; S, *Sal*I; Sc, *Sac*I; X, *Xho*I.

of expression of αTIF during the establishment and maintenance of latent infections were determined.

Construction of MT-αTIF genes. The promoter chosen for these studies was that of the mouse MT-I gene. This promoter is expressed in a wide range of tissues, and its transcription is induced by heavy metals, including cadmium (4, 8). The *Sac*I-to-*Bgl*II restriction enzyme fragment of the MT-I promoter (−147 to +57), which includes the sequences required for heavy metal induction (4), was fused to a DNA fragment containing the αTIF gene from 7 bp upstream of the second ATG (amino acid 12) and including the remaining coding and 3′ transcribed nontranslated sequences (Fig. 1, line 8). This MT-αTIF chimeric gene has been shown to be functional in the induction of HSV α genes in transient expression assays (22a). This construct was also tested and found to be functional in transgenic mice in that the α-TIF transgene could activate expression of an α0-regulated chloramphenicol acetyltransferase transgene upon induction with cadmium (22b). A gene encoding a truncated, nonfunctional MT-αTIF protein (MT-TαTIF) was constructed by inserting a 14-bp double-stranded oligonucleotide containing an *Spe*I restriction endonuclease site and stop codons in all six reading frames into the MT-αTIF gene at the unique *Apa*I site at amino acid 70 (Fig. 1, line 9). If translation of αTIF were to initiate internally, the resulting protein would lack at least the first 75 amino acids (30). Deletion analyses (31a) have shown that proteins lacking amino acids 6 to 63 are greatly reduced in transactivating activity.

Construction of recombinant viruses. We have previously shown (35) that the recombinant virus R314, constructed by insertion of the α4 promoter and other sequences contained in the *Bam*HI Z fragment of HSV-1 into the *Bgl*II site of the *tk* gene, is capable of establishing latent infections in mice and can be reproducibly reactivated from approximately 50% of the trigeminal ganglia of infected mice. To minimize the possibility that changes in the abilities of the viruses tested in these studies to establish latency were due to genomic disruptions, we inserted the MT-αTIF and MT-TαTIF genes into the *Bam*HI Z fragment at the *Kpn*I restriction endonuclease site which is at the opposite end of the fragment from the α4 promoter in pRB314 (Fig. 1, line 11). The DNA fragments containing the MT-αTIF-αTK or MT-TαTIF-αTK construct were recombined into the virus by cotransfection of intact HSV-1(F)Δ305 DNA with the plasmid sequences containing MT-αTIF-αTK (pRB3870) or MT-TαTIF-αTK (pRB3885; Fig. 1, line 10). The progeny of the transfection were then plated on 143TK$^-$ cells in HAT medium to enrich for TK$^+$ progeny. Viruses were plaque purified in 143TK$^-$ cells under HAT medium and then in Vero cell monolayer cultures and were tested for the presence of the insertions by Southern blot analysis of the DNA. The plaque-purified, verified, MT-αTIF and MT-TαTIF viruses selected for these studies were designated R6003 and R6004, respectively (Fig. 1, lines 16 and 17).

Evidence that the MT-αTIF gene is expressed in sensory neurons. Two series of experiments were done to test the capacity of the MT-I promoter used in this study to be expressed in sensory neurons. In the first, the MT-I promoter (−147 to +67) was fused to the *Escherichia coli lacZ* gene, and the chimeric MT-*lacZ* gene was inserted into the gene specifying the viral glycoprotein E. β-Galactosidase activity was detected in sections of trigeminal ganglia of mice latently infected with the recombinant virus (1a). In a more extensive study, mice were infected with R6003 and transcription of the MT-αTIF gene was assayed by in situ hybridization. Mice were infected in both eyes and either not treated or injected with $CdSO_4$ at 15, 17, and 19 days after infection. At 21 days postinfection, trigeminal ganglia were harvested from five untreated and five cadmium-treated mice and assayed for the presence of latent virus. Virus was recovered from 20 and 40% of the ganglia of untreated and cadmium-treated mice, respectively. The remaining mice were then given one more injection of $CdSO_4$ 45 days after infection, and the trigeminal ganglia were harvested 18 h after cadmium treatment and fixed immediately. Sections of ganglia were processed and hybridized with probes complementary to the LAT (Fig. 1, line 2) or to αTIF sequences either complementary to or homologous to the αTIF mRNA (Fig. 1, line 6) as described in Materials and Methods. Ganglionic cells characteristic of neurons and showing autoradiographic grains were readily detected with LAT and αTIF probes (Table 1; Fig. 2) but not with a probe homologous to the αTIF mRNA (Table 1). Table 1 shows the number of neurons scoring positive by hybridization with each probe. Nearly identical ratios of neurons per section hybridizing to probes for LAT and for αTIF mRNA were observed, both in untreated and in cadmium-treated mice. All or most of the latently infected neurons, as distinguished by expression of LAT, were therefore expressing the αTIF mRNA from the MT-I promoter during latent infection.

TABLE 1. Expression of αTIF and LAT by R6003

Virus	No. of positive neurons/no. of sections[a]		
	LAT	αTIF *Mlu*	Anti-αTIF
R6003	10/126	11/124	0/116
R6003 + Cd[b]	10/82	12/78	0/82

[a] Numbers of sections tested are the combined results of two separate hybridization experiments. A total of 8 to 14 sections of each of 20 trigeminal ganglia (R6003) or 14 trigeminal ganglia (R6003 + Cd) were hybridized with each probe.

[b] Mice were injected with $CdSO_4$ (2 mg/ml in saline, subcutaneously in the footpad) 15, 17, 19, and 45 days after infection. Ganglia were removed 18 h after the last $CdSO_4$ injection.

αTIF cannot reactivate latent virus. To determine whether induction of expression of the MT-αTIF gene would lead to lytic infection which would result in ablation of latent virus in trigeminal ganglia, mice were infected in both eyes with R6003 or R6004 and injected with $CdSO_4$ 5, 10, 15, 20, 25, and 30 days after infection (Table 2, experiment 1) or 9, 13, 17, 21, 27, 31, and 34 days after infection (Table 2, experiment 2). In each experiment, control groups of mice infected with either virus were left untreated. No mortality was observed in mice infected with either virus in this or any other experiment. Trigeminal ganglia were harvested 35 (experiment 1) or 37 (experiment 2) days after infection and assayed for the presence of latent virus. No significant reduction was seen in the percentage of ganglia from which virus could be recovered when R6003 was compared with R6004 or when R6003 after cadmium treatment was compared with R6003 with no treatment. Further, there were no differences in the amount of virus which could be recovered from the ganglia of each group of mice (data not shown). All viruses isolated from mice in these experiments were used to infect Vero cell cultures for isolation of viral DNA. Restriction enzyme digests and Southern blot analyses revealed no deletions or rearrangements of the inserted genes (data not shown).

In the second series of experiments, we assessed the capacity of αTIF to reactivate virus after induction by cadmium injection. In these experiments, reactivation was

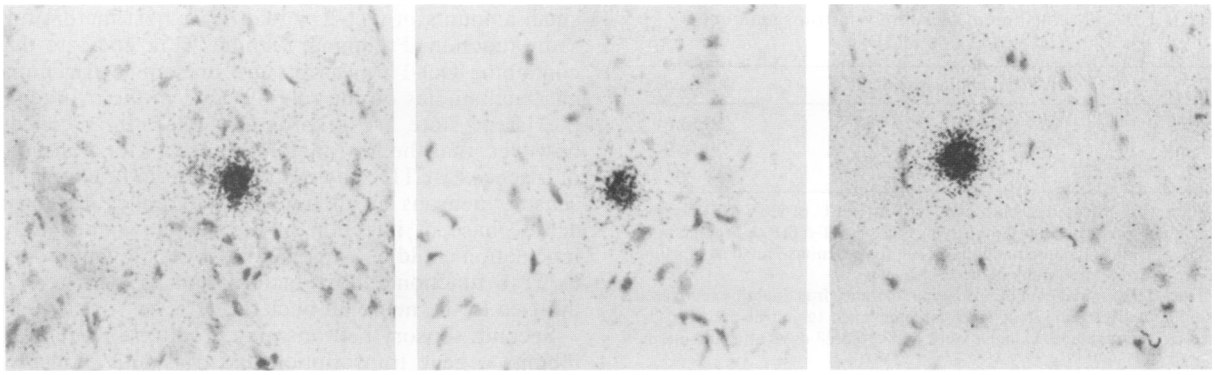

FIG. 2. Photomicrographs of sections of trigeminal ganglia hybridized in situ to a probe specific for αTIF mRNA (600-bp *Asu*II-*Mlu*I transcript complementary to αTIF mRNA; see Fig. 1).

assessed by assaying tears and trigeminal ganglia for the presence of infectious virus after cadmium treatment. In vivo reactivation of HSV in mice by a variety of methods has been reported (11). In the first experiment, 10 mice per virus were infected in both eyes with R6003 or R6004 and were given a single injection of $CdSO_4$ 18 days after infection to induce expression of the MT-I promoter. Eyes were bathed in 10 μl of saline every day for 8 days after the cadmium treatment, and the saline wash was plated on Vero cells. In no case was infectious virus detected.

In the second experiment, 50 mice per virus were infected in both eyes with R6003 or R6004 and given a single injection of $CdSO_4$ 20 days after infection. Trigeminal ganglia were harvested from 10 mice per day at 19 days after infection (before cadmium administration) and at 21, 22, 23, and 24 days after infection (1, 2, 3, and 4 days after cadmium treatment, respectively) and were assayed for the presence of infectious virus. No virus could be detected in any of the ganglia.

Establishment of latency in the presence of αTIF. The lack of reactivation of latent virus in the experiments described above could conceivably have been due to some factor present during the maintenance phase of latency which prevents reactivation and lytic infection after latency has been established but which is not present in appreciable amounts during the establishment of latency. This would apply in particular to any viral gene product which would have to be synthesized after the viral genome reaches the neuronal nucleus. To determine whether expression of αTIF at the time of infection or during the earliest stages of infection could preclude the establishment of latency, two experiments were done.

In the first experiment (Table 3), mice were infected by the eye route with either R6003 or R6004. One group was injected daily with $CdSO_4$ (0 to 7 days after infection) to induce expression of the MT-αTIF and MT-TαTIF genes as soon as the viral genomes arrived at the neuronal nuclei and for several days afterward, while controls remained untreated. Trigeminal ganglia were harvested 12 days after infection and assayed for the presence of latent virus. No significant differences were seen in the percentages of ganglia from which virus could be recovered between any of the four groups of mice (Table 3).

In the second experiment, transgenic mice that contained the same MT-αTIF chimeric gene used to construct recombinant virus R6003 were infected with the wild-type virus, HSV-1(F). These mice have previously been tested and shown to produce functional αTIF protein (22b). Nontransgenic siblings of the transgenic mice were also infected as controls. All of the transgenic mice and one group of the nontransgenic siblings were treated with $CdSO_4$ at the time of infection and daily for 3 days after infection to induce expression of the MT-αTIF gene during the establishment phase of latency. Trigeminal ganglia were harvested 28 days after infection and assayed for the presence of latent virus. Virus was recovered from all three groups of mice in approximately the same amount (Table 4); given the small numbers of mice used, no significant differences were seen in the ability of the wild-type virus to establish latent infections in the transgenic or nontransgenic animals.

TABLE 2. Maintenance of latency after expression of αTIF

Expt	Virus	Inoculum (10^7 PFU/eye)[a]	$CdSO_4$ administration[b]	% Latency[c]
1	R6003	1	None	40 (8/20)
	R6003	1	5, 10, 15, 20, 25, 30	40 (8/20)
	R6004	1	None	10 (2/20)
	R6004	1	5, 10, 15, 20, 25, 30	35 (7/20)
2	R6003	5	None	35 (7/20)
	R6003	5	9, 13, 17, 21, 27, 31, 34	43 (17/40)
	R6004	1	None	20 (4/20)
	R6004	1	9, 13, 17, 21, 27, 31, 34	23 (9/40)

[a] Mice were infected in both eyes following corneal scarification.
[b] $CdSO_4$ was injected subcutaneously (100 μg per mouse in 50 μl of saline) on the days postinfection indicated.
[c] Number of trigeminal ganglia from which latent virus could be recovered/total number of trigeminal ganglia of infected mice (given in parentheses), expressed as a percentage. Ganglia were harvested 35 (experiment 1) or 37 (experiment 2) days postinfection.

TABLE 3. Establishment of latency in the presence of αTIF[a]

Virus	$CdSO_4$ administration[b]	% Latency[c]
R6003	None	33 (6)
R6003	0, 1, 2, 3, 4, 5, 6, 7	44 (8)
R6004	None	38 (7)
R6004	0, 1, 2, 3, 4, 5, 6, 7	17 (3)

[a] Mice were infected in both eyes (10^7 PFU per eye) following corneal scarification.
[b] $CdSO_4$ was injected subcutaneously (100 μg per mouse in 50 μl of saline) on the days postinfection (dpi) indicated.
[c] Number of trigeminal ganglia from which latent virus could be recovered (given in parentheses)/18 trigeminal ganglia from infected mice, expressed as a percentage. Ganglia were harvested 12 days after infection.

TABLE 4. Establishment of latency in transgenic mice expressing αTIF

Mice[a]	$CdSO_4$ administration[b]	% Latency[c]
Wild type	0, 1, 2, 3	50 (3/6)
	None	50 (2/4)
MT-αTIF	0, 1, 2, 3	25 (4/16)

[a] All mice were infected in both eyes with 10^6 PFU of HSV-1(F) per eye. Wild-type mice were nontransgenic siblings of the MT-αTIF transgenic mice.
[b] $CdSO_4$ was injected subcutaneously (100 μg per mouse in 50 μl of saline) on the days postinfection indicated.
[c] Number of trigeminal ganglia from which latent virus could be recovered/total number of trigeminal ganglia of infected mice (given in parentheses), expressed as a percentage. Ganglia were harvested 28 days after infection.

These data indicate that expression of the αTIF at the time of infection and during the early stages of infection is not sufficient to abrogate the establishment of latency by means of a lytic infection.

DISCUSSION

Most people become infected with HSV-1 or HSV-2 by the contact of wet or mucous membranes of susceptible individuals with membranes of individuals with lesions containing virus. These lesions could be the result of first infection but are more likely to be the consequences of reactivation of latent virus. In the latter event, reactivated virus in sensory ganglia is transported to a site at or near the portal of entry of the virus. The reactivated virus is therefore a major source of dissemination of infection, and by extension the latent virus is the reservoir from which the virus is drawn to maintain itself in the human population. Because latency is an important component of the ability of the virus to survive in the human population, we could expect that the processes which regulate the expression of its genes would enable the establishment of latency before viral gene expression committed the cell to a lytic infection, that is, before the expression of α genes. If latency were the result of a failure of αTIF to be transported along with the capsid to the nucleus of the sensory neuron, it would explain many of the unique features of the activation of α gene expression. Specifically, it would explain why α genes utilize a transactivator rather than an enhancer element built into their promoter domains and why αTIF is packaged in association with a structure closely bound to the virion envelope rather than to the viral DNA.

The results presented in this report do not support the hypothesis that latency results solely from the absence of αTIF. Thus, we have not been able to (i) terminate latency by supplying αTIF in *trans* from an endogenous gene linked with a host promoter and embedded in the viral genome, (ii) preclude establishment of latency by the same virus, or (iii) preclude the establishment of latency in transgenic mice expressing αTIF. While these results exclude the absence of αTIF as the sole requirement for the establishment of the latent state, they do not exclude the possibility that a lack of αTIF plays an auxilary role in the process of establishment of latent infection.

Several possibilities remain. First, it has been reported (12) that transcripts of the Oct-1 gene cannot be detected by in situ hybridization in sensory ganglia. However, that study also demonstrated an absence of Oct-1 mRNA in several regions of the central nervous system which are permissive for lytic infection by HSV, leaving open the possibility either that the in situ probes used in that study could not detect small amounts of Oct-1 or that other host factors fulfill the same function. Preliminary studies (15a) indicate that cells containing Oct-1 transcripts are present in trigeminal ganglia, and studies on the role of Oct-1 in the maintenance of the latent state are in progress (32a). It is conceivable, however, that the host factors necessary for transactivation of α genes by αTIF may not be present in functional form in sensory neurons at all times. Viral replication during the acute phase of infection in the ganglia immediately after inoculation could be explained by a very few neurons which express functional host factors and in which αTIF has diffused to the neuronal nuclei.

Second, sensory neurons may produce a repressor which inhibits α gene transcription and transactivation by αTIF. Evidence has been presented that at least one neural cell-derived cell line contains a protein that bound the same sequences bound by Oct-1 and repressed transcription by α gene promoters (19). That cell line, however, was derived from a tumor of central nervous system origin (1), leaving the relevance of those findings to latency in sensory neurons in question inasmuch as the virus grows readily in central nervous system tissue.

Finally, spontaneous reactivation is a sporadic event; only one or a few neurons appear to be reactivated at any one time. This may be an indication that several different events must all take place within a single neuron in order for reactivation of the virus to occur. These events could include the inactivation of a repressor, activation of a host or viral transactivator, or replication of the viral DNA. The experiments described in this study emphasize the necessity for studies of host cell functions as well as those expressed by the virus.

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