Journal of NeuroVirology (1998) 4, 531–538

http://www.jneurovirol.com

Herpes simplex virus latency after direct ganglion virus inoculation

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Herpes simplex virus (HSV) latent infection of ganglion neurons follows axoplasmic transport of HSV, probably in the form of nucleocapsid from peripheral sites of infection (e.g. footpad). This raises the possibility that latency is dependent on this particular means of presenting HSV to ganglion neurons. To investigate this, we directly infected ganglia of mice with HSV and evaluated latency. Initially, ganglia were surgically exposed in intact mice, infected with HSV and after 4 weeks evaluated for HSV latency-associated transcript (LAT) expression. LAT expression suggested latency. To more fully evaluate latency after direct ganglion inoculation, a transplant model was developed. In this model, ganglia were removed from mice, inoculated with HSV, transplanted into syngeneic recipients and evaluated for latency after several weeks. Latency was evident in transplanted ganglia by (1) the presence of LAT in neurons; (2) the lack of HSV ICP4 RNA or viral antigen, and (3) the isolation of HSV from explants of transplants but not from direct homogenates. The transplant model was then used to evaluate the effect of inhibition of HSV replication on latency. Antivirals which inhibited HSV replication markedly decreased the number of LAT-positive neurons in transplants, suggesting a role for HSV replication mechanisms and latency. It is thought that direct ganglion inoculation and ganglion transplant methods will permit unique investigations of mechanisms of latency.

Keywords: viral latent infection

Introduction

Latent herpes simplex virus (HSV) infection is common in human sensory ganglion neurons and HSV latent infection is readily established in sensory ganglia of experimental animals (Ho, 1992; Stevens, 1994; Devi-Rao *et al*, 1997). During latency viral antigen and cell-free virus are not present and the only viral marker readily detected is the HSV latency-associated transcript (LAT). Latent infection in experimental animals has been shown to occur after axoplasmic transport of HSV from the peripheral site of infection to sensory ganglia (Cook and Stevens, 1973; Shimeld *et al*, 1987; Sun *et al*, 1996; Engel *et al*, 1997; Gessner and Koo, 1997). After footpad inoculation of HSV, local colchicine treatment of the sciatic nerve, which inhibits axoplasmic transport, inhibited the establishment of dorsal root ganglion (DRG) latency (Kristensson *et al*, 1971). Similarly, sciatic nerve section blocked the establishment of latency (Tenser *et al*, 1988).

The physical state of HSV and other alpha herpesvirus genomes during axoplasmic transport is probably in the form of viral nucleocapsid (Penfold *et al*, 1994). Since axonal transport of soluble proteins, lipids and organelles occurs at different rates (Grafstein and Forman, 1980; Karlsson *et al*, 1992; Dahlstrom and Li, 1994), it is unlikely that all virion-associated proteins enter the neuronal cell body along with nucleocapsids after axoplasmic transport. Neuron-to-neuron spread may also be axoplasmic (Ugolini *et al*, 1989; Card *et al*, 1991; Whealy *et al*, 1993; Tal-Singer *et al*, 1994; Dingwell *et al*, 1995; Sun *et al*, 1996). The occurrence of axoplasmic spread and the probable disparate delivery of nucleocapsid and viral protein to ganglion neurons raise the possibility that this physical separation is important for the mechanism of establishment of latency in ganglion neurons (Sears *et al*, 1991). In the present study this was investigated by direct HSV inoculation of ganglia.

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Received 20 April 1998; revised 26 May 1998; accepted 27 May 1998

Results of three direct ganglion HSV inoculation studies are presented. In the first, in which transplantation was not utilized, DRG were surgically exposed in intact mice, infected *in situ* with HSV and then tested for latency several weeks later. Based on evidence that latency probably occurred after direct ganglion infection, and in order to permit testing of increased numbers of directly-infected ganglia, a transplant model was established. Normal dorsal root ganglia were removed, infected with HSV, transplanted into syngeneic recipients and subsequently tested for HSV latency. Lastly, the importance of HSV replication on the establishment of latency was evaluated in the transplant model.

Results

Following *in vivo* surgical exposure of the right L-5 DRG and direct inoculation of HSV, latency resulted, as indicated by the expression of LAT 28 days after virus inoculation (Figure 1). Results were positive in four of four mice. Direct *in situ* infection of ganglia required surgical laminectomy, direct visualization and inoculation of the surgically exposed ganglia, extensive postoperative care and then the evaluation of latency 28 days later. Because of the complexity of surgery and animal husbandry, it was not feasible to study large numbers of mice to more completely determine the frequency of LAT expression. However, increased numbers of mice were necessary to determine characteristics of latency and to investigate if persistent rather than latent infection was present. For this reason, an alternative model using transplanted ganglia was investigated. In the transplant model, normal ganglia were removed from mice, infected *in vitro* with HSV and then transplanted beneath the kidney capsule of syngeneic recipients. The establishment of latency was investigated 28–74 days after transplantation. All subsequent studies in the present investigation were performed with this transplant model.

After HSV inoculation of ganglia *in vitro* and transplantation, evidence of acute infection of ganglion transplants was evident for several days, as would be expected. HSV was isolated from homogenates of transplants on day 3, but not on day 7 (Table 1). HSV antigen was detected more frequently in transplants on day 3 than day 7. There was a trend to a greater number of LAT-positive cells on day 7 than day 3. HSV isolation, antigen and LAT results were similar to standard models of acute ganglion infection. The occurrence of acute infection in transplants supported plans to investigate latency in transplants.

Latency in DRG transplants 28 days after transplantation was defined by: (1) the presence of LAT; (2) the lack of ICP4 RNA; (3) the lack of HSV antigen; (4) the isolation of HSV from explants of transplants and (5) the lack of isolation of HSV from direct homogenates of transplants (Table 2). LAT was detected (Figure 2) on day 28 in 12 of 16 transplants (75%) with an average of 31 LAT-positive neurons per LAT-positive transplant and ICP4 RNA was not detected in any of eight transplants studied

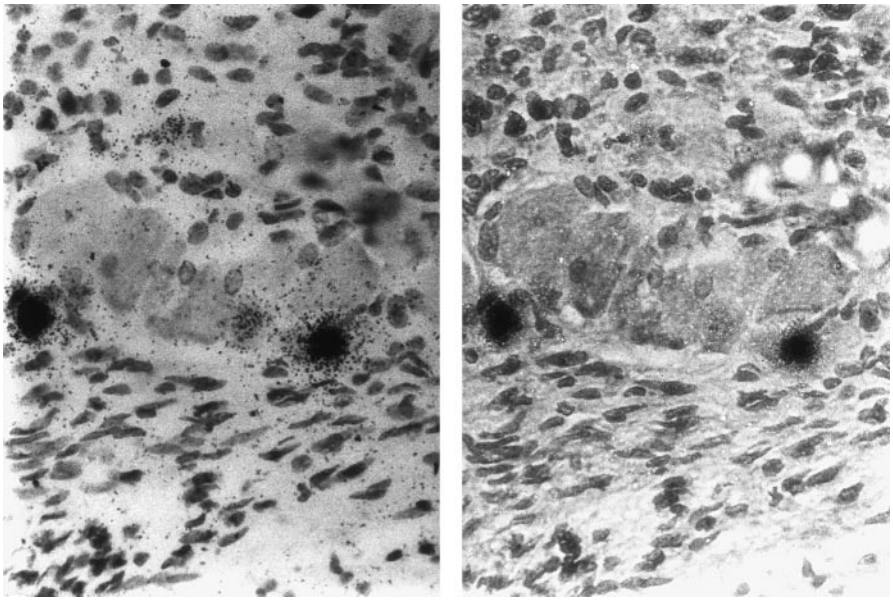

Figure 1 HSV LAT expressed in L-5 DRG neurons 28 days after direct *in situ* ganglion inoculation with HSV. The DRG was surgically exposed by a laminectomy. After 28 days the mouse was sacrificed and the DRG processed for LAT detection by *in situ* hybridization. Left, standard light microscopy. Right, phase contrast.

Table 1 HSV acute infection in DRG transplants.[a]

Time after HSV inoculation of DRG and transplantation, days	*Titer of HSV in homogenates of transplants (number tested)*	*HSV antigen; Number positive transplants/total (%)*	*HSV LAT-positive transplants; Number positive/total (%)*	*Average number LAT-positive neurons[b]*
3	3.2×10^4 (3)	3/4 (75)	2/5 (40)	2
7	0[c] (3)	2/4 (50)	6/6 (100)	12

[a]Normal DRG were removed, infected *in vitro* and after incubation for 1 h transplanted into syngeneic recipients. After 3 or 7 days transplanted DRG were removed and assayed.
[b]Average number of LAT-positive neurons in LAT-positive transplants.
[c]Level of detection was 5 PFU per ganglion.

Table 2 HSV latency in DRG transplants.

Time after HSV inoculation and transplantation days[a]	*Isolation of HSV; number positive transplants/number tested (%)*		*Detection of HSV antigen; number positive transplants/number tested (%)*	*Detection of ICP4 RNA; number positive transplants/ number tested (%)*	*Detection of LAT; number positive transplants/ number tested (%) [average number LAT-positive neurons][b]*
	Homogenates	*Explants*			
28	0/7 (0)	7/10 (70)	0/7 (0)	0/8 (0)	12/16 (75) [31]
31–33	0/6 (0)	ND[c]	0/4 (0)	0/4 (0)	6/6 (100) [94]
63–74	ND	ND	ND	ND	6/6 (100) [82]

[a]Ganglion removal, infection and transplantation as in Table 1. Latency was evaluated at times noted.
[b]Average numbers of LAT-positive neurons in LAT-positive transplants.
[c]Not done.

(Table 2). In a methodological control of acutely infected trigeminal ganglion tissue, ICP4 RNA was readily detected (results not shown) as previously reported (Tenser *et al*, 1994). The lack of ICP4 RNA in transplants suggested that acute or persistent infection was not present. This was further supported by the lack of HSV antigen in these transplants. In HSV isolation studies, after explant culture of transplants, HSV was isolated from seven of 10 transplants. However, HSV was not detected in any of seven transplants directly homogenized without an explant period (Table 2). The lack of infectious HSV in direct homogenates further suggested that acute or persistent HSV infection was not present and the occurrence of reactivation in explants of transplants indicated typical HSV latency. Taken together, these results suggested that HSV latency was established in this model of direct inoculation of HSV into DRG followed by transplantation.

Similar to day 28 results, evidence of latency was evident 31–33 days after transplantation. Specifically, ICP4 RNA was not detected in transplants in which LAT was readily detected. There were greater numbers of LAT-positive cells on days 31–33 than on day 28, and the increase continued to be present on days 63–74 (Table 2). Results showing latency more than 2 months after transplantation indicated the viability of substantial numbers of neurons.

The model of direct inoculation of DRG followed by transplantation was then used to investigate the importance of HSV replication and latency. Antivirals were used to inhibit HSV replication and numbers of LAT-positive neurons in transplants then determined. In initial studies without antiviral use, (Table 2) HSV was inoculated into normal ganglia and after incubation *in vitro* for 1 h, ganglia were washed and transplanted. In this antiviral study, after the 1 h period, ganglia were incubated in the presence of the antiviral acyclovir for an additional hour. In addition, antiviral-treated ganglia were transplanted into antiviral-treated mice. These mice received both acyclovir intraperitoneally and then famciclovir in their drinking water. The goal of this study was to directly evaluate the effect of inhibition of HSV replication on the establishment of latency. Twenty-eight days after transplantation, DRG transplants were removed and processed for *in situ* hybridization detection of HSV LAT. All transplants were LAT-positive (eight of eight control and eight of eight antiviral treated). However, the number of LAT-positive neurons was markedly decreased in the antiviral group (Table 3). An average of 3 LAT-positive neurons were detected in the antiviral-treated group and 80 in the untreated control group. This suggested very inefficient establishment of latency in the antiviral group.

Discussion

The occurrence of latency in transplants indicated that axoplasmic transport of HSV from periphery to ganglion neurons was not necessary for the establishment of HSV latency. It is concluded that HSV virion and other components present in the HSV inoculum did not prevent latency. Since the usual route of HSV presentation to ganglion neurons, i.e. axoplasmic transport, was not necessary in order for latency to be established, the present results re-emphasize the importance of neuronal factors in the development of latency. The lack of latency in other than neurons cannot be explained by a requirement for axoplasmic transport, which might alter the presentation of HSV. Mechanisms of infection of neurons following direct DRG inoculation with HSV remain to be determined. It is likely that usual methods of ingress of HSV (Fuller and Lee, 1992; Spear, 1993) are necessary, since direct ganglion inoculation with HSV nucleocapsids (prepared by the treatment of virions with lipid solvents) did not result in latency (data not shown).

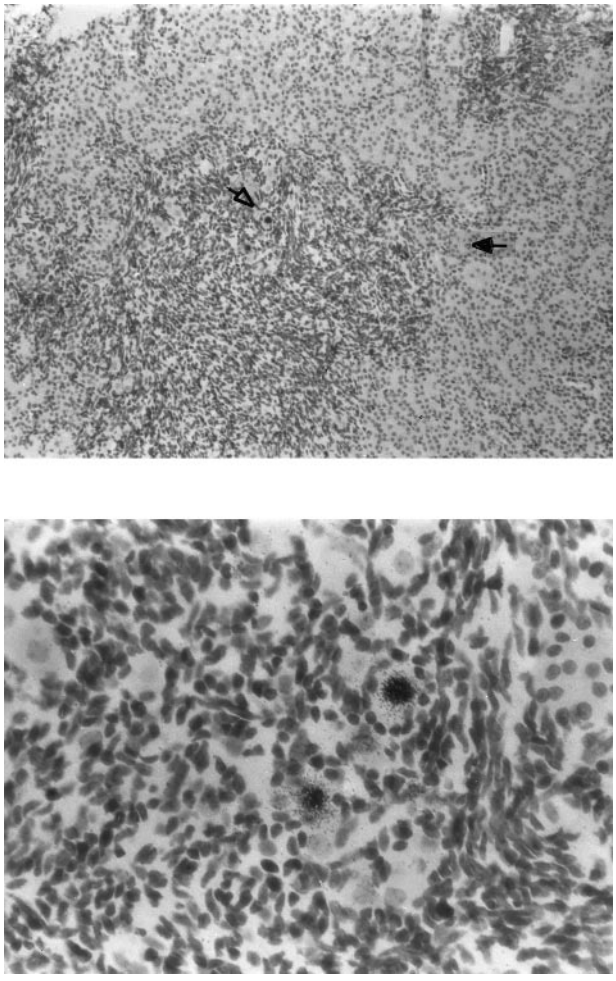

Figure 2 HSV LAT expressed in DRG 28 days after HSV inoculation and transplantation. Normal DRG were removed, infected *in vitro* and transplanted. After 28 days the syngeneic recipient was sacrificed and the DRG transplant processed for LAT detection. Top, low power magnification showing the transplant (closed arrow) with two LAT-positive cells (open arrow) and adjacent kidney tissue. Bottom, high power magnification of the two LAT-positive neurons in the transplant.

It was previously shown that transplanted ganglia survived sufficiently well to investigate mechanisms of HSV latency. In that study, latently-infected DRG were removed and transplanted. Latency was maintained in transplants for several weeks. Based on several experiments it was concluded that reactivation of HSV occurred in transplants and that latent infections were then established in the neurons which had not been previously latently infected. This was termed secondary latency (Tenser *et al*, 1994). These transplant results led to plans to study direct HSV infection of normal DRG followed by transplantation, as was done in the present investigation. It is thought that formal criteria of latency were met in the present study and that HSV latent infection was established in transplants. The development of latency after direct inoculation of HSV into ganglia is provisionally termed virion latency. The terms secondary latency and virion latency are used only in an operational sense and do not imply differences in latency pathogenesis.

The direct ganglion HSV inoculation results suggested the occurrence of latency although the efficiency and/or quantitative development of latency remain unclear. Quantitation of latency is difficult in all models since quantitation may be determined by ganglion HSV LAT or HSV DNA, number of LAT-positive neurons or number of reactivation competent neurons (Maggioncalda *et*

Table 3 Effect of inhibition of HSV replication on latency in DRG transplants.[a]

	Time after HSV inoculation and transplantation, days	*No. LAT-positive ganglion transplants/number tested (%)*	*Average number LAT-positive neurons per transplant*
Control, untreated	28	8/8 (100)	80
Antiviral treated	28	8/8 (100)	3

[a]Normal DRG were removed from mice, infected with HSV and incubated for 60 min at 37°C. After 1 h, infected ganglia were washed and incubated in medium containing acyclovir for 1 h, or in standard medium. Ganglia were then transplanted into syngeneic recipients. Mice in the antiviral group received intraperitoneal administration of acyclovir and then famciclovir in their drinking water for 9 days.

al, 1996; Lekstrom-Himes *et al*, 1998). At the present it is only possible to present a qualitative conclusion, that direct ganglion inoculation can result in latency.

In the transplant model, proximal intraganglionic axon segments remained, although disconnected from normal peripheral axon processes. It can be speculated that HSV entered remaining axon segments and then entered neuronal cell bodies. This is thought unlikely, but cannot be completely ruled out. What was most directly eliminated by direct ganglion inoculation was local epithelial replication of HSV at a peripheral site of HSV inoculation and then axoplasmic transport of HSV over a distance. Experiments to further limit possible intraganglionic axoplasmic transport might be attempted by combining direct inoculation and use of drugs such as colchicine. Such drugs have been shown to be effective in inhibition of the establishment of latency *in vivo* when applied locally to peripheral nerve (Kristensson *et al*, 1971). In the present transplant model, however, systemic whole animal treatment would be necessary and would likely be toxic if used in appropriate amount.

After establishment of the transplant model, the model was utilized to investigate the role of HSV replication on the establishment of latency. Following transplantation, LAT was detected in both untreated and antiviral-treated ganglia. However, numbers of LAT-positive cells were markedly decreased in antiviral-treated transplants. These results suggested a role for HSV replication in latency, although the presence of some LAT-positive neurons in antiviral-treated transplants does not permit an unequivocal conclusion. In mice treated with antivirals and sacrificed 3 days after transplantation, HSV was isolated in 50% of mice in very reduced amount (data not shown). This suggested either residual input virus or more likely low-level virus replication. Therefore, antiviral treatment inhibited but did not completely block HSV replication in transplant recipients treated with antivirals (Table 3). Treatment with a greater amount of drug might have eliminated HSV detected on day 3 and further inhibited latency. Only if latency were completely blocked with an antiviral would there be an unequivocal indication of the necessity for viral replication and the establishment of latency. In studies using thymidine kinase mutants of HSV, which are defective for replication in neurons, decreased replication correlated with decreased numbers of LAT-positive cells (Coen *et al*, 1989; Tenser *et al*, 1994). However, in studies with more generally replication-defective HSV mutants, the establishment of latency was reported (Steiner *et al*, 1990; Sedarati *et al*, 1993), which suggested that HSV replication was not necessary for latency. Differing results suggesting that HSV replication is or is not important for the establishment of latency remain to be explained.

The transplant model may be generally useful to investigate latency, in part since time of ganglion infection is more definitively known than after usual peripheral (i.e. footpad) inoculation. With footpad inoculation local HSV replication occurs and the relative contributions of input inoculum virus and subsequent HSV replication to axonal HSV transport are unclear. In the transplant model it is likely that inoculum HSV resulted in latency. Furthermore, after direct HSV inoculation of DRG, ganglia can be maintained *in vitro* for up to 48 h prior to transplantation. Latency is detected (i.e., LAT is expressed) in such delayed transplants (data not shown). During the *in vitro* period prior to transplantation, it may be possible to modify the infection in several ways and to alter development of latency.

Materials and methods

Viruses and mice

HSV (HSV-type 1, strain KOS) (Tenser *et al*, 1994) was grown and titrated in Vero cells by standard methods using medium 199 supplemented with 2% fetal calf serum. Stock titers were 10^8 PFU ml^{-1} Balb/c mice (Charles River Laboratories, Wilmington, MA) 6–8 weeks old were housed and cared for in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Direct infection of DRG in intact mice

Mice were deeply anesthetized (pentobarbital; 1.5 mg/mouse). Fur over the lumbosacral region was removed and the skin washed with 70% ethanol. Superficial and deep dissection was performed with scalpel and scissors under sterile conditions to expose the lumbar-4 (L-4) to L-6 vertebral bodies. The left L-5 vertebral lamina was carefully removed through the use of scissors and bone rongeurs, to expose the L-5 DRG. The left L-5 DRG was then directly inoculated with 1 μl of stock HSV. Subcutaneous tissues were closed with nylon sutures and the skin with sutures or wound clips. Surgery was performed on a warmed surface (heating pad), and mice were kept warm after surgery until recovery from anesthesia.

In vitro *infection of dorsal root ganglia prior to transplantation*

L-4, L-5 and L-6 DRG were surgically removed under general anesthesia (methoxyflurane) from normal Balb/c mice. After being briefly washed in sterile balanced salt solution, DRG were infected with HSV-1 by placing them in wells of a 96-well plate with 10^6 PFU in a volume of 0.1 mL. The DRG were punctured 4–5 times with a sterile 25-gauge

needle. After incubating the DRG with virus for 60 min at 37°C, the DRG were washed three times in medium.

Transplantation of infected ganglia
HSV-infected DRG were transplanted beneath the renal capsule of syngeneic recipients, as previously described (Tenser *et al*, 1994). For this procedure, recipients were anesthetized (methoxyflurane) and the fur on the left posterior flank was removed. The left kidney was exposed through a posterior incision under sterile conditions. The kidney capsule was nicked with a scalpel, and transplants were gently inserted beneath the capsule. Peritoneal and cutaneous tissues were closed with surgical clips. Three DRG (one L-4, one L-5 and one L-6) were transplanted together. In all discussion below, each group of three ganglia will be referred to as a transplant. At several times points after transplantation, mice were sacrificed and transplanted ganglia were assayed for acute and latent HSV infection.

HSV acute infection in transplants
On day 3 and 7 after infection and transplantation, transplanted ganglia were assayed for acute infection. Acute infection assays included the detection of viral antigen and the isolation of HSV in homogenates of transplanted ganglia. Acutely infected ganglia were also tested for HSV LAT expression by *in situ* hybridization.

HSV latent infection in transplants
On day 28–74 after HSV infection and transplantation, transplanted ganglia were assayed for evidence of latent infection. Evidence of latency required all of the following: HSV LAT by *in situ* hybridization, lack of HSV ICP4 RNA by *in situ* hybridization, lack of HSV antigen, isolation of HSV in explants of transplants but not in direct homogenates of transplants.

Methods of detection of HSV
Infectious HSV in homogenates of transplanted DRG Kidneys bearing transplants were removed from anesthetized and exsanguinated mice and transplants were dissected free from surrounding kidney tissue. Ganglia were frozen at −70°C in 0.5 ml of medium containing 2% FCS. After homogenization and centrifugation, supernatants were cultured on Vero cell monolayers to isolate infectious HSV. Isolation of HSV in direct homogenates indicated the presence of acute infection.

HSV antigen in transplanted DRG Transplants were removed and fixed with 4% paraformaldehyde and paraffin sections were prepared. HSV antigen was detected with polyclonal HSV antibody (1:500; Dako, Santa Barbara, CA) using peroxidase-antiperoxidase procedures described previously (Tenser *et al*, 1994). Detection of HSV antigen indicated the presence of acute infection.

Isolation of HSV in explants of transplanted DRG Transplants were dissected free from surrounding kidney and explanted at 37°C in 0.5 ml medium for 6 days. After that period, ganglia were homogenized and assayed for infectious virus on Vero cell monolayers. Detection of infectious virus after explantation of transplants but not in direct homogenates of transplants was considered to indicate latent infection.

HSV LAT in situ *hybridization* HSV-1 LAT was detected by *in situ* hybridization on serial sections (10 μM) by hybridization with a strand-specific oligonucleotide as previously described (Tenser *et al*, 1994). In brief, a synthetic oligonucleotide, corresponding to nucleotides 611 to 640 of the HSV-1 sequence for LAT, was 3′ end-labeled ([35S]-dATP; 1000 Ci/mmole; NEN). Tissue sections were hybridized with the labeled probe for 48 h to 72 h at 37°C in 10% dextran sulfate, 45% formamide and 2 × SSC [1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate]. Individual LAT-positive neurons were counted after 6 days exposure to liquid emulsion (NTB-3, Eastman Kodak Co., New Haven, CT).

HSV ICP4 RNA in situ *hybridization* HSV ICP4 RNA was investigated by *in situ* hybridization by techniques as for LAT. An oligonucleotide probe corresponding to nucleotides 1722 to 1757 of the published sequence (McGeoch *et al*, 1986) was used, as previously described (Tenser *et al*, 1994). Acutely infected trigeminal ganglion tissue was used as a positive control.

Inhibition of LAT expression in DRG transplanted by acyclovir and famciclovir
In some experiments viral replication in transplanted DRG was inhibited using acyclovir and famciclovir. Normal DRG were infected *in vitro* as before, but after incubation and washing, the infected DRG were placed in medium containing acyclovir (50 μg ml^{-1}) for 1 h (37°C) until transplantation. Control HSV inoculated ganglia were incubated in standard medium. In addition, transplant recipient mice in the antiviral (acyclovir) group were injected intraperitoneally with 1 mg acyclovir dissolved in 0.1 ml sterile saline at the time of transplant. Control mice received 0.1 ml saline. The same antiviral (acyclovir) recipients were also given famciclovir (1 mg ml^{-1}) in their drinking water, which also contained sucrose (5%), for 9 days following transplantation. It was previously noted that mice drink 9–

10 ml of nucleoside-sucrose water per day (Tenser *et al*, 1996). Control mice received 5% sucrose water. Twenty eight days after transplantation the number of LAT-positive neurons in transplants was determined by *in situ* hybridization.

Acknowledgements

The secretarial assistance of Tricia Spitler is greatly appreciated. Investigations were supported in part by grant PP0548 from the National Multiple Sclerosis Society.

References

Card JP, Whealy ME, Robbins AK, Moore RY, Enquist LW (1991). Two α-herpesvirus strains are transported differentially in the rodent visual system. *Neuron* **6:** 957–969.

Coen DM, Kosz-Vnenchak M, Jacobson JG, Leib DA, Bogard CL, Shaffer PA, Tyler KL, Knipe DM (1989). Thymidine kinase-negative herpes simplex virus mutants establish latancy in mouse trigeminal ganglia but do not reactivate. *Proceedings of the National Academy of Science* **86:** 4736–4740.

Cook ML, Stevens JG (1973). Pathogenesis of herpetic neuritis and ganglionitis in mice: Evidence for intra-axonal transport of infection. *Infection and Immunity* **7:** 272–288.

Dahlstrom AB, Li JY (1994). Fast and slow axonal transport-different methodological approaches give complementary information: contribution of stop-flow/crush approach. *Neurochemical Research* **19:** 1413–1419.

Devi-Rao GB, Aguilar JS, Rice MK, Garza HH, Bloom DC, Hill JM, Wagner EK (1997). Herpes simplex virus genome replication and transcription during induced reactivation in the rabbit eye. *Journal of Virology* **71:** 7039–7047.

Dingwell KS, Doering LC, Johnson DC (1995). Glycoproteins E and I facilitate neuron-to-neuron spread of herpes simplex virus. *Journal of Virology* **69:** 7087–7098.

Engel JP, Madigan TC, Peterson GM (1997). The transneuronal spread phenotype of herpes simplex virus type 1 infection of the mouse hind footpad. *Journal of Virology* **71:** 2425–2435.

Fuller AD, Lee W-C (1992). Herpes simplex virus type 1 entry through a cascade of virus-cell interactions requires different roles of gD and gH in penetration. *Journal of Virology* **66:** 5002–5012.

Gessner RM, Koo SC (1997). Latent herpes simplex virus type 1 gene expression in ganglia innervating the human gastrointestinal tract. *Journal of Virology* **71:** 4103–4106.

Grafstein B, Forman DS (1980). Intracellular transport in neurons. *Physiology Review* **60:** 1167–1283.

Ho DY (1992). Herpes simplex virus latency: Molecular aspects. *Progress in Medical Virology* **39:** 76–115.

Karlsson JO, Inomata M, Kawashima S (1992). Slow axonal transport of soluble proteins and calpain in retinal ganglion cells of aged rabbits. *Neuroscience Letters* **141:** 127–129.

Kristensson K, Lycke E, Sjostrand J (1971). Spread of herpes simplex virus in peripheral nerves. *Acta Neuropathology* **17:** 44–53.

Lekstrom-Himes JA, Pesnicak L, Straus SE (1998). The quantity of latent viral DNA correlates with the relative rates at which herpes simplex virus types 1 and 2 cause recurrent genital herpes outbreaks. *Journal of Virology* **72:** 2760–2764.

Maggioncalda J, Mehta A, Su YH, Fraser NW, Block TM (1996). Correlation between herpes simplex virus type 1 rate of reactivation from latent infection and the number of infected neurons in trigeminal ganglia. *Virology* **225:** 72–81.

McGeoch DJ, Dolan A, Donald S, Brauer DHK (1986). Complete DNA sequence of the short repeat region in the genome of herpes simplex virus type 1. *Nucleic Acids Research* **14:** 1727–1745.

Penfold MET, Armati P, Cunningham AL (1994) Axonal transport of herpes simplex virus to epidermal cells: Evidence for a specialized mode of virus transport and assembly. *Proceedings of the National Academy of Science* **91:** 6529–6533.

Sears AE, Hukkanen V, Labow MA, Levine AJ, Roizman B (1991). 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. *Journal of Virology* **65:** 2929–2935.

Sedarati F, Margolis TP, Stevens JG (1993). Latent infection can be established with drastically restricted transcription and replication of the HSV-1 genome. *Virology* **192:** 687–691.

Shimeld C, Dyson H, Lewkowicz-Moss S, Hill TJ, Blyth WA, Easty DL (1987). Spread of HSV-1 to the mouse eye after inoculation in the skin of the snout requires an intact nerve supply to the inoculation site. *Current Eye Research* **6:** 9–12.

Spear PJ (1993). Entry of alphaherpesviruses into cells. *Seminars in Virology* **4:** 167–180.

Steiner I, Spivack JG, Deshmane SL, Ace CI, Preston CM, Fraser NW (1990). A herpes simplex virus type 1 mutant containing nontransinducing Vmw65 protein establishes latent infection in vivo in the absence of viral replication and reactivates efficiently from explanted trigeminal ganglia. *Journal of Virology* **64:** 1630–1638.

Stevens JG (1994). Overview of herpes latency. *Seminars in Virology* **5:** 191–196.

Sun N, Cassell MD, Perlman S (1996). Anterograde, transneuronal transport of herpes simplex virus type 1 strain H129 in the murine visual system. *Journal of Virology* **70:** 5405–5413.

Tal-Singer R, Eisenberg RJ, Valyi-Nagy T, Fraser NW, Cohen GH (1994). N-linked oligosaccharides on herpes simplex virus glycoprotein gD are not essential for establishment of viral latency or reactivation in the mouse eye model. *Virology* **202:** 1050–1053.

Tenser RB, Edris WA, Hay KA (1988). Herpes simplex virus latent infection: reactivation and elimination of latency after neurectomy. *Virology* **167:** 302–305.

Tenser RB, Edris WA, Gaydos A, Hay KA (1994). Secondary herpes simplex virus latent infection in transplanted ganglia. *Journal of Virology* **68:** 7212–7220.

Tenser RB, Gaydos A, Hay KA (1996) Reactivation of thymidine kinase-defective herpes simplex virus is enhanced by nucleoside. *Journal of Virology* **70:** 1271–1276.

Ugolini G, Kiypers HGJM, Strick PL (1989) Transneuronal transfer of herpes virus from peripheral nerves to cortex and brainstem. *Science* **243:** 89–91.

Whealy ME, Card JP, Robbins AK, Dubin JR, Rziha H-J, Enquist LW (1993). Specific pseudorabies virus infection of the rat visual system requires both gI and gp63 glycoproteins. *Journal of Virology* **67:** 3786–3797.