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Review

Herpes simplex virus latent infection in the nervous system

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Herpes simplex virus (HSV) establishes a latent infection in the human peripheral nervous system and can cause recurrent disease by reactivation. Intensive effort has been directed in recent years to unveil the molecular, cellular and immune mechanisms, as well as the virus–host interactions associated with latent HSV infection. The aim of this review is to summarize current knowledge regarding the site of latent infection, the molecular phenomena of latency, and the mechanisms of the various stages of HSV-1 latent infection in the nervous system, relating them where possible to the human situation. Specifically, the following biological questions are addressed: (1) How does this lytic virus survive in the nervous system and why can it establish a lifelong latent infection in nerve cells? (2) What advantage is conferred on HSV by establishing latent infection in nervous tissue? (3) What can be gathered from the accumulated knowledge on latency about the pathogenesis of herpes simplex encephalitis?

Keywords: herpes simplex virus; latent infection; viral pathogenesis; nervous system; gene expression

Introduction

The ability of herpes simplex virus type 1 (HSV-1) to colonize and establish latent infection in the human nervous system has attracted much scientific interest ever since Goodpasture postulated in 1929 that the recurrent mucocutaneous disease stems from a dormant viral state in the peripheral sensory ganglia (PSG). Half a century later, Stevens and colleagues (Stevens and Cook, 1971; Cook and Stevens, 1973) as well as other groups (Bastian *et al*, 1972; Baringer and Swoveland, 1973) established the validity of this hypothesis by isolating HSV from human PSG. Recent years have witnessed a plethora of data on the molecular concomitants of HSV latency (for reviews see Roizman and Sears, 1987; Stevens, 1989; Steiner and Kennedy, 1993) and the information so gathered in this field has now paved the way for the use of HSV-derived vectors as vehicles for gene therapy in neurological disorders (Kennedy and Steiner, 1993). While it is possible that there are sites of extraneural HSV latency (Clements and Subak-Sharpe, 1988; Abgharis and Stulting, 1988; Stevens, 1978; Kaye *et al*, 1991), it is widely accepted that HSV latency is an almost exclusive feature of nervous tissue.

The aim of this review is to summarize current knowledge on HSV-1 latent infection and to examine its relevance to human disease. Specifically, we will address the following questions: (1) By what mechanisms does this lytic virus survive in the nervous system and how can it establish a lifelong latent infection in nerve cells since the usual outcome of most viral infections is either destruction of the host, or elimination of the viral pathogen from the organism? (2) What is the relevance of the accumulated experimental data on latency to the pathogenesis of HSV-1-induced disease in humans? (3) What advantage is conferred on the virus by establishing latency in nervous tissue? In this rather controversial field, considerable differences may exist

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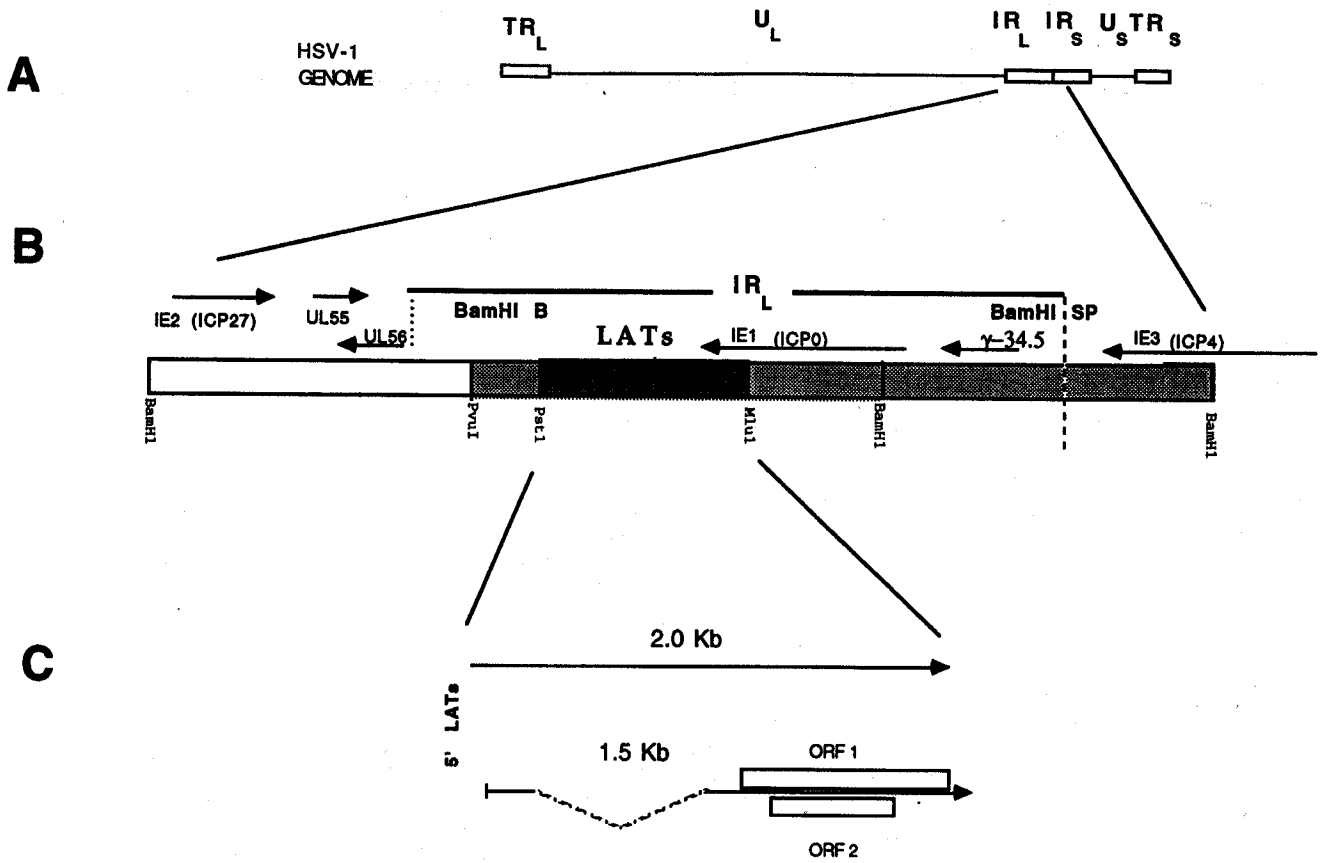

Figure 1 Herpes simplex virus type 1 (HSV-1) genome organization, location and structure of the viral latency-associated gene expression. (a) The HSV-1 genome illustrating the unique long (U_L) and unique short (U_S) regions bounded by the terminal and internal repeat regions (TR and IR respectively). (b) Detailed map of BamHI restriction fragments B and SP. The approximate location of the immediate early (ICP4, ICPO and ICP27) and other (γ-34.5, UL55 and UL56) genes' mRNAs clustered in this fragment of the viral genome are marked by arrows that indicate their transcription orientation. The region of the viral genome that is transcriptionally active during HSV-1 latent infection and is positive by *in situ* hybridization is shaded. The darkly shaded region between PstI and MluI restriction sites gives rise to two overlapping HSV-1 LATs. Also denoted are the location of the internal repeat long (IR_L) and the border between the long and short fragments of the genome. (c) Map and structure of the LATs. The 5′ and 3′ ends of the LATs, and the location of the open reading frames (ORFs) are illustrated.

in the individual researcher's interpretation of some of the data. While attempting to provide an overview of HSV latency, we have inevitably formulated our own views which do not necessarily conform with current established dogmas.

Background

Definition of latency

Latent infection is defined as the presence of the viral genome in the tissue without production of infective viral particles. However, the virus maintains the potential to reactivate, resume replication and cause recurrent disease. In the case of HSV-1, molecular criteria must be added to this definition since, as will be discussed, during latency, the structure of the viral DNA and the pattern of its gene expression differ from the situation present during viral replication in cell culture.

Animal models

The greater volume of information pertaining to latency of the neurotropic herpesviruses stems from studies with HSV-1 in experimental animals. Subsequently the data were substantiated on human tissue and findings were also later extrapolated to studies using HSV-2 (Mitchell *et al*, 1990) and varicella-zoster virus (VZV) (Croen *et al*, 1988). The available models share a similar basic approach (Fraser *et al*, 1984): peripheral (eg cornea, pinna of the ear, etc) inoculation results in transport of viral particles to the respective sensory ganglia and to the central nervous system (CNS) where replication takes place. When replication ceases, the animal tissues may be examined for the various aspects of the latent state. The animal models made it possible to break down the continuous process of HSV-1 latency into several stages which can be

studied separately: (1) viral replication at the peripheral site of infection; (2) transport of viral particles to the nervous system; (3) establishment of latent infection; (4) maintenance of the latent state for the entire life of the host; and (5) reactivation.

Viral structure and replication in cell culture
The entire double stranded 152 kb DNA genome of HSV-1 (Figure 1a) has been sequenced (McGeoch *et al*, 1986; 1988; Perry and McGeoch, 1988) and most of its gene products have been characterized for structure and function (Roizman and Sears, 1990). The mature virion includes a tegument layer located between the capsid and the envelope. Replication of herpes DNA and the assembly of the viral capsid take place within the nucleus of the infected cell during a highly coordinated process with temporal synthesis of viral gene products according to function (for reviews see Roizman and Sears, 1987; 1990). HSV-1 genes are roughly divided into three groups: immediate early (IE) or α genes which are activated by a tegument structural protein Vmw65 (Batterson and Roizman, 1983; Campbell *et al*, 1984); genes which encode for enzymes involved in nucleotide metabolism (early, β genes), that are activated by α genes; and late (γ) genes, which mainly code for structural proteins. During productive HSV-1 infection the infected cells undergo major structural and biochemical alterations that ultimately culminate in their destruction. However, it has been suggested that the expression of α genes alone may not be deleterious to the host (Roizman and Sears, 1987; Morhan *et al*, 1989).

Primary infection and establishment of latency

How does the viral genome reach the nervous system?
Following peripheral inoculation and viral replication, HSV-1 DNA can be detected in the respective innervating PSG. However, HSV-1 replication at the peripheral site of primary infection is not a prerequisite for the ability of HSV-1 to reach the PSG (Efstathiou *et al*, 1989; Steiner *et al*, 1990). With or without peripheral replication, the timing of viral DNA appearance at the PSG suggests that following virion attachment to axonal terminals it is the fast retrograde axonal flow which mediates viral transport to the nerve cell body (Cook and Stevens, 1973; Kristensson *et al*, 1986).

The prevention of HSV-1 replication as a key to the establishment of latency
Once HSV-1 reaches the PSG, it can resume replication. However, replication will cause host cell destruction while factors, viral or cellular, which will prevent replication will enable the cell to survive and host the latent viral genome in the tissue, thus favouring the establishment of latency. Indeed, prevention of replication by passive immunization prior to ocular infection results in decreased detectable peripheral replication of virus, but increases the amount of viral DNA and the number of latently infected neurons in PSG via an unknown mechanism (Birmanns *et al*, 1993). It is noteworthy, therefore, that while most human adults are seropositive for HSV, and up to 61% of them have recurrent mucocutaneous disease, only less than 5% have a history of clinical primary disease (Whitley, 1985), suggesting that viral replication may not take place during all primary HSV-1 infections. Several factors can prevent replication or abort it prior to host cell destruction. Most of them are not an absolute prerequisite for the ability of HSV-1 to establish latent infection, and some of them may act in concert to enable the establishment of latency.

Viral factors which favour establishment of latency
Vmw65 may play a pivotal role in the outcome of HSV-1 infection and the ability of the virus to enter a latent state (Steiner *et al*, 1990; Steiner and Kennedy, 1991). During attachment of HSV-1 virions to the axonal membrane, the virus loses its envelope, and therefore, part of the content of the tegument, Vmw65 included, may be lost during viral transport to the neuronal nucleus (Lycke *et al*, 1984, 1988). The amount of Vmw65 reaching the nucleus is one determinant of whether a lytic replication cycle will ensue. Indeed, lack of functional Vmw65 was associated with higher proportion of latently infected neurons (Steiner *et al*, 1990). However, the presence of Vmw65 by itself does not prevent the establishment of latency (Sears *et al*, 1991). Another factor which might influence the ability of HSV-1 to replicate and therefore affect the formation of a latent infection, is the amount of inoculated virus (Fraser *et al*, 1991). This is analogous to the ability to overcome a defect in replication of a mutant virus in cells in culture by increasing the multiplicity of infection (MOI). It might be of special relevance in certain cells, eg neurons, which possess mechanisms to prevent HSV-1 replication and are therefore relatively non-permissive for HSV-1 replication.

The non-permissiveness of neurons for HSV-1 replication as favouring the establishment of latent infection
Several reports suggested that neurons, at least in culture, express factors which have an inhibitory effect upon expression of HSV-1 IE genes (Ash, 1986; Kemp *et al*, 1990; Wheatley *et al*, 1991; Lillycrop *et al*, 1993), and therefore arrest HSV-1 replication at an early stage prior to irreversible cell damage. One of these factors is Oct-2, a transcrip-

tional factor expressed at high levels in nerve cells. By binding to a protein complex containing Vmw65 it can inhibit the IE activation activity of Vmw65 (Lillycrop *et al*, 1993). Thus, increasing the MOI will diminish the repressor activity of Oct-2 by increasing the relative amount of Vmw65 and leading eventually to initiation of the HSV-1 replication cascade.

The role of the immune system

Immunization of mice with hyper immune serum prior to primary infection, prevents replication and increases the number of latently infected neurons (Birmanns *et al*, 1993). Several mechanisms might be responsible for this observation: antibodies can bind to HSV-1 infected neurons and downregulate intracellular viral replication (Oaks and Lausch, 1984), and CD8+ T cells can terminate viral gene expression in neurons without cytolysis (Simmons and Tscharke, 1992). However, latent infection can be formed in immunodeficient mice (Moriyama *et al*, 1992; Valyi Nagy *et al*, 1992), suggesting that while the immune mechanisms might contribute to the establishment of latent HSV-1 infection they are not absolutely required.

Molecular phenomena of HSV-1 latency

DNA structure, location and organization

The entire HSV-1 genome is present in latently infected nervous tissue, but the ends within the terminal repeats (Figure 1a) are present in amounts suggesting that the latent HSV-1 DNA is maintained either as a circular molecule or in a concatameric form (Rock and Fraser, 1983, 1985; Efstathiou *et al*, 1986). The viral DNA is not integrated into the host cell genome (Mellerick and Fraser, 1987), and is organized in a structure similar in pattern to host nuclear chromatin (Deshmane and Fraser, 1989). The amount of viral DNA per latently infected cell is unknown but it might be relevant to the mechanisms of latency and reactivation, since it has been suggested (Roizman and Sears, 1987; Fraser *et al*, 1991) that a smaller number of viral DNA copies per cell is associated with inability to initiate replication within the nucleus and a larger copy number will facilitate reactivation.

Gene expression

No infectious viral particles are present during latent infection. Therefore, the discovery of latent phase HSV-1 transcriptional activity has stimulated intensive research aimed at mapping and characterizing HSV-1 latency-associated genes and elucidation of their function. The viral latency-associated transcription stems from a 10.4 kb fragment within the repeat regions (Figure 1b) (Stevens *et al*, 1987; Deatly *et al*, 1987; Croen *et al*, 1987; Steiner *et al*, 1988; Gordon *et al*, 1988; Stevens *et al*, 1988), but only a 2.0 kb subfragment synthesizes two mRNAs, 2.0 and 1.5 kb in size, which are abundant enough to be studied by Northern blot analysis (Spivack and Fraser 1987; Steiner *et al*, 1988; Krause *et al*, 1988). These latency-associated transcripts (LATs) are transcribed in the opposite direction to the IE gene ICPO (Figure 1b–c) and overlap its mRNA3 end by approximately 700 bp. Only the 2.0 kb LAT can be identified during viral replication in cell culture, and in much smaller amounts than those present during latency (Spivack and Fraser 1987; Krause *et al*, 1988; Spivack and Fraser, 1988b). The LATs have several unique features: (1) they do not belong to any of the three viral gene classes as characterized during viral replication in cell culture (Spivack and Fraser, 1988b); [56] (2) they accumulate during latency (Spivack and Fraser, 1988a, b) and by *in situ* hybridization can be demonstrated mainly or exclusively on and around the nucleus of the infected cell (Stevens *et al*, 1987; Croen *et al*, 1987; Steiner *et al*, 1988); (3) their promoter is located at an unusual distance from their coding sequence start site (Batchelor and O'Hare, 1990; Dobson *et al*, 1989; Zwaagstra *et al*, 1989, 1990), and its activity is increased in neuronal cells in culture (Batchelor and O'Hare, 1990, 1992; Zwaagstra *et al*, 1990; Devi-Rao *et al*, 1991). Recently, another 'cryptic' promoter at a closer location to the initiation of the LATs coding sequence was reported (Nicosia *et al*, 1993; Goins *et al*, 1994); (4) the 2.0 kb LAT transcript is unspliced, ie no RNA fragments (termed introns) are removed to form it (Figure 1c). The 1.5 LAT is a spliced product derived by removal of a 0.5 kb intron (Figure 1c) (Wagner *et al*, 1988b; Wechsler *et al*, 1988; Spivack *et al*, 1991), with a nucleic acid sequence at the splicing donor site of GC instead of the consensus GT (Mount, 1982). It may therefore serve as an inefficient splice signal during viral replication in cells in culture and non-neuronal tissue (Spivack *et al*, 1991) and be responsible for neuronal-specificity of production of the 1.5 kb LAT (Mador, Panet and Steiner, submitted for publication); (5) the 2.0 kb LAT, has a splice donor site around the beginning of its coding sequence (Devi-Rao *et al*, 1991; Farrel *et al*, 1991), suggesting that it is a stable intron. However, nuclear accumulation is not a characteristic of introns and it is very unusual for introns to be further processed.

Besides the LATs, additional RNAs stem from the latency-associated 10.4 kb transcriptionally-active region within the HSV-1 genome (Figure 1b), but their relation to the LATs is unclear. *In vitro* studies suggested the presence of two unstable larger transcripts, 8.3–8.5 and 6.5 kb in size (Dobson *et al*, 1989; Zwaagstra *et al*, 1990; Devi-Rao *et al*, 1991). The LATs are mainly unpolyadenylated (Spivack and Fraser, 1987; Wagner *et al*, 1988a), ie the several adenine nucleotides at the end of the mRNA molecule required for its transport to the cytoplasm and

the translation into protein are missing. On the other hand, the unstable larger transcripts, when examined during viral replication in cell culture, are polyadenylated (Devi-Rao *et al*, 1991).

Gene products
No latent-phase HSV-1 gene products (LATs-coded or other) have been identified *in vivo* so far. At present, the possibility that no polypeptides are encoded by the latency-associated genes seems plausible despite the fact that at least two nucleic acid sequences that can code for a sequence of amino acids are present within the LATs (Figure 1c) (Wechsler *et al*, 1989; Spivack *et al*, 1991).

Function and mechanisms of action of the latency-associated genes

Function
A number of HSV-1 mutants defective in their ability to express LATs have been used to examine the function of the latency-associated transcriptional activity (Javier *et al*, 1988; Steiner *et al*, 1989; Ho and Mocarski, 1989; Lieb *et al*, 1989; Sedarati *et al*, 1989; Hill *et al*, 1990; Trousdale *et al*, 1991). These mutant viruses retain the ability to replicate in tissue culture and to establish and maintain a latent infection *in vivo*. However, a consistent viral phenotype is associated with some LAT-defective viruses: aberrant and prolonged explant reactivation and severely reduced ability for *in vivo* reactivation. While this may suggest that the LATs participate in the reactivation process, it is also possible that the reduced reactivation ability of LATs(-) mutants may be a consequence of establishment of latency in fewer initial sites (Sawtell and Thompson, 1992). Recent data has also shown that in latently infected mouse PSG, LATs and IE genes (the latter acting as a marker of HSV-1 reactivation) are not coexpressed by the same cells (Ecob-Prince *et al*, 1993a, b).

Mechanisms of HSV-1 reactivation
HSV reactivation in humans, with resultant cold sores or genital lesions, can be triggered by local stimuli (such as injury to tissues innervated by the neurons harboring latent infection) or by systemic conditions including exposure to sun, fever, emotional stress and menstruation (Hill, 1985). Even after repeated bouts of reactivation, most individuals do not exhibit permanent sensory loss or any other neurological deficit in the affected dermatomes (Gominak *et al*, 1990). We therefore assume that reactivation is not associated with a significant destruction of latently infected neurons and thus differs from HSV-1 lytic replication.

Two major molecular questions which might be relevant to the mechanisms of action of the latency-associated genes are open at this point: (1) How does replication begin in the absence of Vmw65 protein to stimulate the early stages of the HSV-1 replication cycle? (2) How does the viral genome get to the stage of active replication at the periphery without prior destruction of the cell hosting the latent viral genome?

Viral factors Theoretically the LATs, could augment and facilitate reactivation at four different points: (1) Increasing the amount of latent viral DNA during the establishment of latency. This is supported by a study that demonstrated more latently infected cells in TG of mice infected with wild-type virus than with a LATs(-) mutant, suggesting that the LATs may in fact participate in establishment of latency (Sawtell and Thompson, 1992). (2) Releasing, or counteracting, under certain permissive circumstances, a neuronal inhibitory factor that renders the neuron relatively non-permissive to HSV-1 replication during the maintenance of latency. However, since even recurrent reactivations are not accompanied by sensory loss, it seems unlikely that reactivation is associated with HSV-1 replication in the PSG. (3) Initiation, at the periphery, of the replication cascade of HSV-1. Since Vmw65 is not expressed during latency, it seems that a crucial step in the ability of HSV-1 to reactivate is the presence of some viral or cellular factors which circumvent the initial lack of Vmw65 to activate IE gene expression. Such a function might be mediated by the latency-associated transcription. (4) Facilitating the transfer of the viral nucleic acids from the PSG via the axon to the periphery where the virus can resume replication and reactivation without damaging the nerve cells and the viral reservoir. The fact that reactivation with LATs(-) mutants, though defective, is possible with explant tissues (Steiner *et al*, 1989) (where axonal transport is not required) but not *in vivo* (Hill *et al*, 1990; Trousdale *et al*, 1991), may support the notion that LATs have a role in viral DNA transport.

As no lantency-associated gene product has been identified as yet, it is possible that these genes act via functional RNA. The end of the LATs overlaps with that of ICP0 and therefore an antisense mechanism (whereby a homologous transcript of opposing orientation binds to a mRNA and physically blocks its translation into gene products) for the LATs was proposed (Stevens *et al*, 1987). While this hypothesis could already be discounted on theoretical grounds, results with HSV-1 LATs(-) mutants have excluded it experimentally: the reactivation kinetics of these mutants is not stimulated as it should have been in the case of the absent antisense inhibition of ICP0, but, on the contrary, is impaired.

Cellular factors The effect of cellular factors upon reactivation and LATs expression has been examined *in vitro* and has yielded two interesting findings. (1) Nerve growth factor (NGF) may have a role in rendering a neuron non-permissive for viral reac-

tivation, since it is required for the maintenance of a quasi-latent form of HSV in a neuronal cell tissue system (Wilcox and Johnson, 1987; Wilcox *et al*, 1990). (2) The promoter region of the LATs contains a cAMP-responsive element and administration of cAMP accelerated explant reactivation via a sequence located within the LAT promoter region (Lieb *et al*, 1991; Rader *et al*, 1993). In this case, extraneural stimuli might trigger HSV reactivation via second messenger signal transduction and cAMP action upon LATs regulatory elements.

Immune factors Immunosuppression of latently infected animals leads to HSV-1 reactivation (Birmanns *et al*, 1993; Openshaw *et al*, 1979), suggesting that immunological mechanisms are associated with maintenance of latency. However, this phenomenon is the exception, since in humans, recurrent disease usually occurs in immunocompetent hosts who are seropositive for HSV-1. Moreover, latency is maintained under conditions associated with defective T- and B-cell functions (Moriyama *et al*, 1992; Valyi-Nagy *et al*, 1992). As no viral antigens are expressed during latent infection, and the process of reactivation probably does not involve host cell destruction within the PSG, immune responses are unlikely to be evident in the PSG. On encountering mature virions at the periphery, however, the immune system may be activated.

CNS latency and the source of the virus causing encephalitis

Although HSV-1 is responsible for the viral encephalitis with the highest fatality rate, much less is known about the molecular biology of latent HSV-1 infection in the CNS than in the PNS. HSV has also been implicated in the pathogenesis of other CNS disorders such as multiple sclerosis (Kastrukoff *et al*, 1987), Behçet disease (Eglin *et al*, 1982) and Alzheimer's disease (Ball, 1982), but no conclusive evidence to link HSV (both 1 and 2) with any CNS disease other than meningoencephalitis, myelitis (Shyu *et al*, 1993) and Mollaret's meningitis (Yamamoto *et al*, 1991; Cohen *et al*, 1994) has been produced so far.

HSV-1 reactivation ability from CNS tissue is extremely limited

Following viral replication within TG in experimental animals the virus travels to, and replicates in, the CNS (Stevens, 1989), but when replication ceases, explanted brainstem tissues do not reactivate virus. Similarly, despite the presence of HSV-1 nucleic acids in the human brain (Fraser *et al*, 1981, and our unpublished data) we are unaware of any report of *recurrent* encephalitis induced by HSV-1, and attempts to reactivate HSV-1 from explanted human CNS tissue have failed. Herpes encephalitis in immunocompetent individuals is a very rare single event which is 1 million fold less frequent than the peripheral disease (Whitley, 1985). The source of the virus causing herpes encephalitis is not known. It has been suggested that encephalitis is due to HSV-1 reactivation from TG (Johnson, 1982), since the infectious process tends to involve the temporal and frontal lobes, brain regions with blood vessels and meninges which derive their sensory innervation from the TG. However, since not all cases of herpes encephalitis are caused by the same viral strain that is responsible for cold sores in the same individual (Whitley *et al*, 1982), it is assumed that in approximately half of the patients HSV-1 encephalitis occurs during primary viral infection (Whitley, 1990). Moreover, even in the presence of a prior HSV-1 infection, a second primary infection with another HSV-1 strain can take place and thus may be responsible for the encephalitic infection.

Several factors may be responsible for the differences between HSV-1 reactivation from the peripheral and the central nervous systems. (1) The quantity of the LATs may contribute to the efficacy of reactivation (Birmanns *et al*, 1993; Block *et al*, 1990). While the organization of the HSV-1 DNA in the brainstem and in the TG is similar (Rock and Fraser, 1983), the relative amounts of viral DNA and latency-associated gene expression in the CNS are lower than those in the PNS (Steiner *et al*, 1994). (2) The type of gene expression in the CNS may differ from that present in PSG. Thus, the 1.5 kb LAT was not detected in brainstem tissue (Steiner *et al*, 1994). (3) Still unknown host and CNS-specific tissue factors might inhibit viral reactivation in the CNS.

A unifying scheme—the advantage of latency in nervous tissue for the virus (Figure 2)

We suggest the following sequence of events. During primary infection, HSV-1 enters sensory nerve terminals at the peripheral site of inoculation and is transported to the nerve cell body and nucleus via fast retrograde axonal transport. In the nucleus of the neuron, prevention of lytic infection is critical for the establishment of latency. Several factors, cellular and viral, can act alone or in concert to prevent replication and enable the establishment of latency. Recent evidence even suggests that neurons may have a unique ability to survive viral infections by blocking programmed cell death (Levine *et al*, 1993). Both the establishment of latency and the latency-associated restricted gene expression take place at a very early stage, following arrival of the viral genome in the nucleus.

The unique ability of neurons to transport molecules away from their cell body to other tissues and distant body regions, and from the periphery into the neuronal soma, has an obvious advantage for

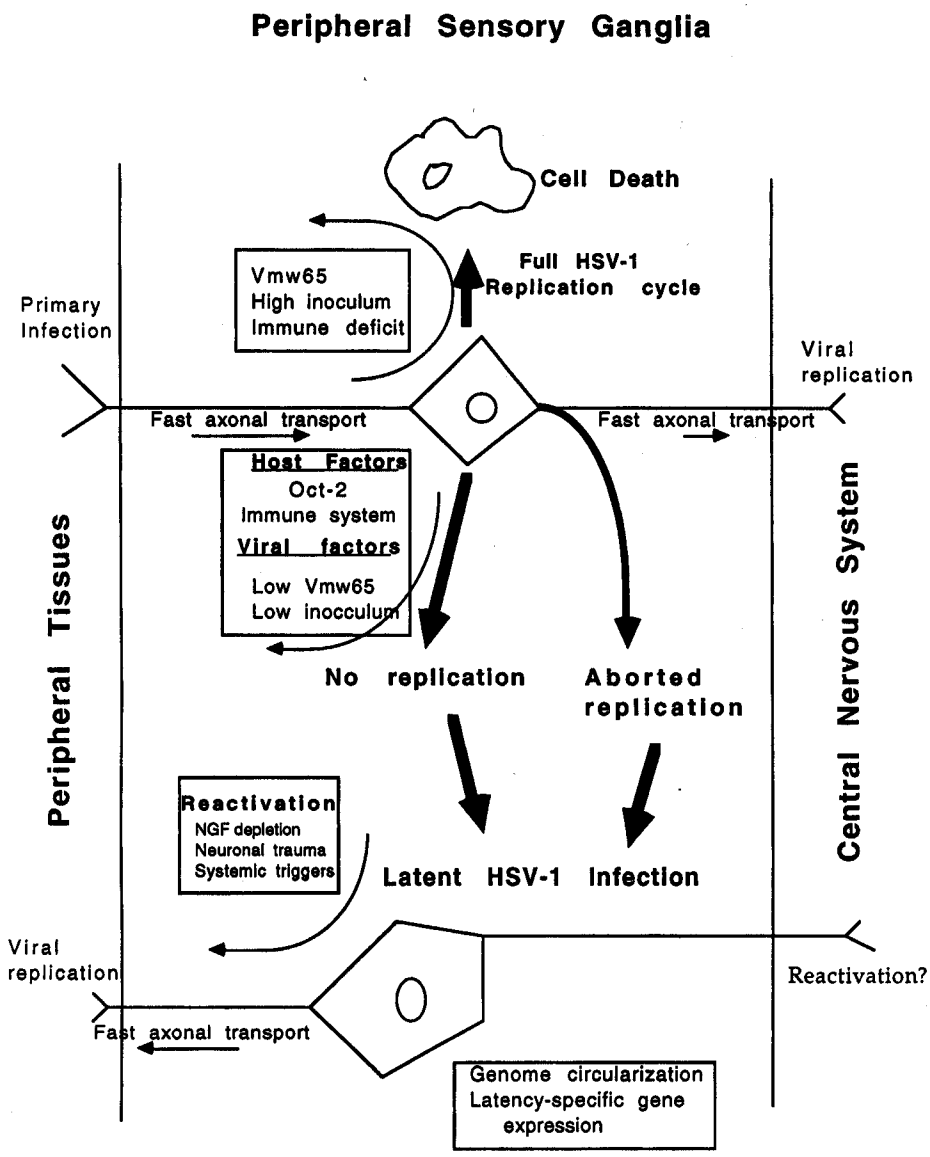

Figure 2 Schematic illustration of herpes simplex virus replication cycle and possible mechanisms of latent infection in the nervous system of animals. Following primary infection at peripheral mucocutaneous tissues, the virion travels via fast axonal transport to peripheral sensory ganglia (PSG). Under certain conditions which favour viral replication, such as high levels of Vmw65, high amount of inoculated virus and defective immune mechanisms, a full replication cycle will ensue, leading to neuronal cell death. By constrast, an effective immune system, low levels of Vmw65, low amounts of inoculated virus and neuronal-specific factors such as Oct-2 will prevent viral replication and could lead to the establishment of latent infection. Neurons are also capable of aborting HSV replication at a stage which will not lead to cell death and enable the formation of latent HSV-1 infection. Following primary infection, HSV is also able to travel to the central nervous system (CNS) and replicate there. During latency HSV-1 genome is 'endless' and latency-specific gene expression takes place. Under specific systemic and local triggers the latent genome can reactivate, travel to the periphery and replicate there. The ability of HSV-1 to reactivate and travel to the CNS is uncertain.

the survival of HSV-1. It makes it possible physically to separate the site of active viral replication in the mucocutaneous tissues from the neuronal cell body, which serves as the permanent reservoir of viral DNA, and remains undamaged. Viral replication in the peripheral mucocutaneous tissues enables the spread of infection to other carriers. While it is possible that viral DNA is transported from PSG to the CNS as well as to the periphery, reactivation of HSV-1 DNA does not occur in the CNS (unlike replication, when a replication competent virus reaches CNS tissue). It is therefore likely that HSV-1 encephalitis is due either to primary infection in a rare case of a seronegative individual,

or due to an infection with another, HSV-1 strain, rather than reactivation of latent virus from the trigeminal ganglia.

The role of the HSV-1 LATs in reactivation is not entirely clear. The LATs might act to stimulate host functions required for reactivation, or conversely, to inhibit the synthesis of host factors that suppress reactivation. Alternately, they may replace Vmw65 protein function and initiate IE expression. Another possibility is that the LATs may promote the migration of latent viral DNA out of the neuronal nucleus, into the cytoplasm and the axon. Further research is required to establish the role of the latency-associated gene expression in the HSV-1 latency process, and we anticipate significant advances in the understanding of HSV latency at the molecular level over the next few years. The extent to which such advances will enhance our understanding of HSV-associated neurological diseases such as HSV encephalitis remains uncertain.

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