

HERPES SIMPLEX VIRUS: A TOOL FOR NEUROSCIENTISTS

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1. ABSTRACT

Herpes viruses have received a great deal of attention due to their widespread and ubiquitous prevalence in the human population and to the diverse range of diseases caused as a result of an infection. During the last 20 - 25 years, many research laboratories have investigated the pathogenesis and molecular biology of these viruses; particularly herpes simplex virus (HSV). As a result of this research, HSV has begun to get the attention of neuroscientists. In fact, in the last few years there has been an explosion of research involving the use of HSV and related viruses as tools or model systems for different areas of neuroscience research. This brief review will describe several of these areas including demyelinating diseases, neuronal tracings, and genetic therapy.

2. INTRODUCTION

In order to understand the different roles of HSV in neuroscience, it is necessary to have a general understanding of the different types of viral infections and the replicative cycle.

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2.1. HSV infections

Infection with HSV can result in several diseases ranging from inapparent infections and self-limiting cutaneous lesions to fatal encephalitis (for a review, see 1). In a primary infection, HSV enters the body via mucosal membrane or abraded skin and establishes a local infection in epithelial cells. Viral replication in these cells results in the amplification of virus, the formation of a 'fever blister', and the activation of both cellular and humoral immune responses. During this acute infection, the virus is transported by retrograde axonal transport to the nuclei of the sensory neurons innervating the site of the local infection (2). Studies using animal models have indicated that a limited viral replication occurs within these neurons followed by the establishment of latency.

A latent infection is characterized by the presence of viral genomes (in the nuclei of sensory neurons) and the absence of viral replication or viral protein production (for review, see 3). The infection and establishment of latency within neurons explain why HSV is termed a neurotrophic virus. In a latently-infected neuron, virus-specific proteins are not produced and, as a result, the host's immune system is unaware of the virus' presence and does not target the latently-infected neuron for destruction. Latent infections ensure the survival and persistence of the virus in the human population.

A latent HSV infection is maintained for the life of the host, but the virus can be reactivated periodi-

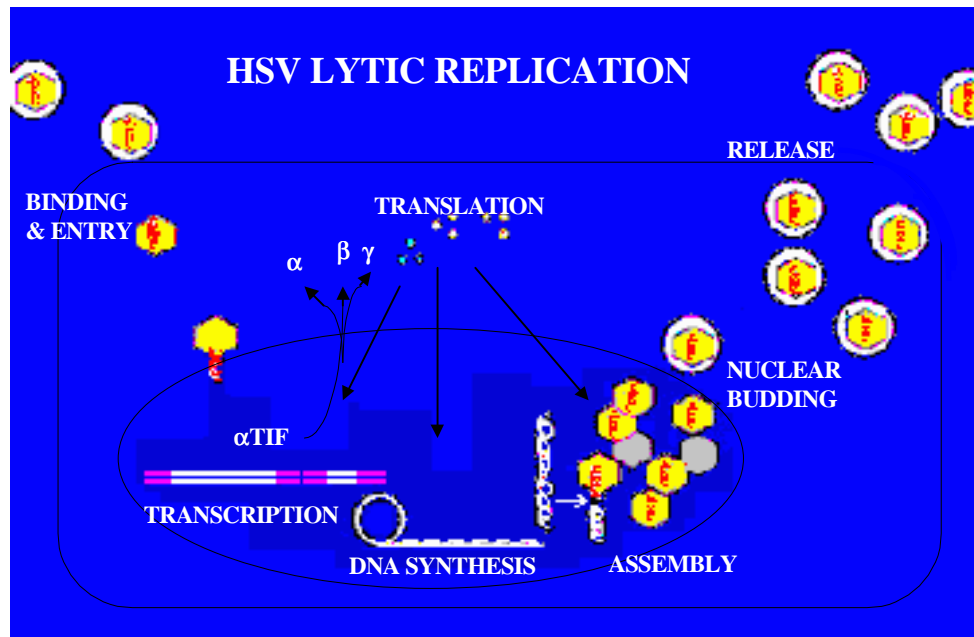


Figure 1. Schematic representation of an HSV lytic replication cycle.

cally to produce infectious virus and recurrent disease. During reactivation, the viral genome in the latently-infected cell is activated resulting in viral replication. The reactivated virus then travels down the sensory axon where it establishes an infection in the epithelia of the skin. Studies using both animal models and human subjects have shown that viral reactivation can be triggered by a variety of stressful or stress-related stimuli including heat, U.V. light, fever, hormonal changes, menses and physical trauma to the neuron (*e.g.* 4-7). While the virus appears to be latent most of the time, HSV infection is probably best characterized by recurrent reactivations and periods of latency.

2.2. HSV replicative cycle

HSV is a large, enveloped virus that contains an icosahedral nucleocapsid and a amorphous structure termed the tegument located between the nucleocapsid and envelope. For the purposes of this review, we will briefly review the general replication scheme of HSV (depicted in Figure 1). For a detailed review, the reader is directed to Roizman and Sears (8). The enveloped virus particle binds to the outside of a susceptible cell resulting in a fusion between the viral envelope and cellular membrane. As a result of membrane fusion, the nucleocapsid enters the cell cytoplasm and migrates to the nuclear membrane. The viral genome is released from the capsid structure and enters the nucleus through nuclear pores. Once inside the nucleus, viral-specific transcription, translation, and replication of the DNA genome occur. The newly synthesized viral DNA is packaged into preformed capsid structures and the nucleocapsid buds through the nuclear membrane, obtaining its envelope. The replication of HSV is fairly quick, occurring within 15 hours post-

infection and is extremely lethal to the cell resulting in cell lysis.

The genome of HSV is a linear, double-stranded DNA molecule approximately 152 KB in length that encodes for a minimum of 75 separate proteins (9). HSV genes are divided into three temporal classes (α , β , and γ) which are regulated in a coordinated, cascade fashion (for review see 8). The α or immediate-early (IE) genes contain the major transcriptional regulatory proteins and their production is required for the transcription of the β and γ gene classes. Of the 5 immediate early genes identified, ICP4 represents the major regulatory protein of HSV. The synthesis of ICP4 is absolutely required for viral replication and this protein is involved in the transactivation of both β and γ genes. The β proteins consist primarily of proteins involved in viral nucleic acid metabolism and are not produced in the absence of α proteins. The synthesis of the β proteins precedes and is required for replication of the viral DNA genome. The γ proteins consist primarily of virus structural proteins and their synthesis occurs after the onset of viral DNA replication. Molecular studies on a majority of the genes encoded by the HSV genome have demonstrated that many of them can be deleted without interfering with the virus' ability to replicate in cell culture lines (10). In addition, it is possible to construct site-specific mutations, including the deletion of viral genes and the insertion of foreign genes, into the viral genome (11, 12).

The properties of HSV that make it a useful tool for studies in the field of neuroscience include its neurotropism, the ability to construct viral mutants and its ability to establish latent infections in neuronal cells.

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In this review we will briefly describe several roles for HSV in neuroscience including 1) a model for demyelinating disease, 2) a tool for transneuronal tracing studies, and 3) use as a viral vector for gene therapy.

3. DEMYELINATING DISEASE

3.1. CNS vs PNS infections

Animal studies have demonstrated that during an acute infection, HSV spreads from peripheral epithelial cells to both the peripheral nervous system (PNS) and central nervous system (CNS; 13-17). Interestingly, cellular damage induced by the virus is markedly different between the PNS and CNS. Tissue damage in the PNS is generally mild while the damage in the CNS is often extensive within a local foci. A well studied example of this concept is seen within the trigeminal root entry zone (TREZ) of the brainstem, a junction region between the PNS and CNS (13, 14, 18). Within this region, following an acute HSV infection, the peripheral myelin is untouched while the CNS side of the trigeminal root develops demyelinated lesions. Within the demyelinated lesions, there is an absence of myelin and the presence of both intact axons and a mononuclear cell (MNC) infiltrate. The exact mechanism of demyelination is not known, but the results from several studies suggest that it is a combination of cellular infection and host immune response.

3.2. Role of immunosuppression

Glucocorticoid-induced immunosuppression has been reported to reduce immune cell infiltrate and myelin destruction in the CNS portion of the TREZ following peripheral HSV infection (14) and demyelinating lesions were absent in nude (athymic) mice inoculated corneally with HSV (19). A recent study comparing TREZ demyelination following peripheral HSV infection in immunocompetent mice and immune deficient mice, and immunocompetent mice immunosuppressed with glucocorticoids, demonstrated that immune deficient or immunosuppressed mice exhibited reduced levels or no demyelinating lesions (20). These studies demonstrate the role of the host's immune response in demyelination. In addition, other studies have indicated that HSV travels from the site of peripheral infection to the PNS and then to the CNS where it infects and lyses astrocytes. The infection and subsequent lysis of astrocytes occurs prior to the appearance of demyelination (21, 22). Thus HSV-induced demyelination may also be due, in part, to a cytotoxic effect of virus replication in astrocytes and oligodendrocytes in the CNS.

The role of immunopathology in HSV-induced CNS demyelination is one reason why HSV infection serves as a useful model for studies investigating human demyelinating diseases such as Bell's Palsy and multiple sclerosis (23). In addition, studies on the mechanism of CNS demyelination and long-term effects of

demyelination may serve as good models for trigeminal neuralgia (24).

4. TRANSNEURONAL TRACERS

Neuroscientists have long desired to map chains of neurons in order to identify communication pathways from origin to termination. With the use of antero- and retrograde tracers such as fast blue and horseradish peroxidase it is possible to identify single neurons along with their axons and terminations. However, in order to identify synaptically linked second and third order neurons, transneuronal tracers are required.

4.1. Properties of effective transneuronal tracers

In order for a transneuronal tracer to be effective, it must be specific for synaptically-linked connections, possess the ability to be transported antero- or retrogradely and be sufficiently tagged for efficient and sensitive detection. Substances such as cholera toxin, tetanus toxin and wheat germ agglutinin are known to bind specifically at neuronal membranes and have been used as transneuronal tracers (25-27). These methods, however, have limitations since only small amounts of protein are transported across synapses resulting in an absence of or relatively weak labeling of linked neurons. In addition, nonspecific labeling of adjacent neurons can occur at increased injection concentrations and extended labeling times. Neurotropic viruses, specifically herpesviruses, have an advantage over these other contemporary methods in that they are able to replicate within neuron cell bodies providing signal amplification before infecting second- and third-order neurons. These viruses have also been shown to specifically label neuronal connections in both the retrograde and anterograde direction (28, 29).

4.2. Viral transneuronal tracers

The most common transneuronal viral tracers are herpes simplex virus 1 and 2 (HSV-1/HSV-2) and pseudorabies virus (PRV). All three viruses belong to the alpha herpesvirinae family and therefore are neurotrophic (8). The ability of these DNA viruses to specifically infect neurons contributes to their specific transneuronal transport. The most common method used to detect the presence of these viruses in neuronal tissue is by immunohistochemical staining for viral antigen.

Experiments used to obtain transneuronal tracings are modulated by the strain of virus used, the host animal, the site of injection, the amount of virus inoculated, and the time of post-inoculation analysis. The importance of these parameters is apparent from many studies which report that uncontrolled viral tracings, especially at late survival times, may lead to nonspecific labeling (30, 31).

Electron microscopic studies using HSV and PRV have demonstrated that fusion of the viral envelope

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with the cellular plasma membrane of neuronal extensions is followed by retrograde axonal transport of unenveloped nucleocapsids along axonal microtubules (28). Although this is the primary mode of viral transport to the neuronal nucleus, it is not exclusive. Other studies have shown anterograde transport of virus, (29, 32) and at least one report suggests that the direction of transneuronal transport may be strain dependent (33). By analyzing labeled neurons at progressive time points, it has been determined that retrograde transport occurs much faster than anterograde transport (29). Consideration of the difference in transport rate is important in tracing analyses and can be useful in determining connections between groups of neurons. For example, in groups of neurons which are highly connected by collaterals, one must consider the fact that individual neuron labeling could be due to either anterograde or retrograde transport, and in such instances, there may be no way to distinguish between originating and target cells.

Although the release of herpes virus occurs at neuronal terminals, sites of virion egress do not always occur directly into synaptic clefts. Herpes-containing vesicles have been reported to fuse at presynaptic terminals releasing enveloped virus which then fuses to postsynaptic membranes adjacent to the presynaptic terminals resulting in the entry of nucleocapsids into the neuron. Astrocytes are also susceptible to PRV and HSV infection, but infected cells are only observed subsequent to an adjacent neuronal infection. Ultrastructural analyses of PRV-infected astrocytes have revealed a defect in the cytoplasmic envelopment of viral nucleocapsids rendering the nucleocapsids incapable of plasma membrane fusion. This defect results in an absence of viral egress and an accumulation of virion particles within the cellular cytoplasm (34). The resulting abortive infection effectively prevents astrocytic PRV virions from contributing to nonspecific extracellular spread. At present, no such mechanisms are known for HSV. In fact, several studies have reported that HSV is quite capable of establishing a productive infection in astrocytes (35). The inability of PRV to establish a productive infection in astrocytes provides a great advantage to PRV in ensuring specific transneuronal transport and is a major reason why PRV is considered by many to be the virus of choice for CNS transneuronal tracing studies.

Additional host mechanisms restricting the spread of the virus to non-neuronal cells are provided by the host's immune response to both PRV and HSV infections. Resident microglia, monocytes and macrophages are activated in the nervous system during viral infection and may effectively phagocytose virus and degenerating cellular debris (36). The importance of these mechanisms is apparent considering the large viral load which may be released from necrotic cells to the extracellular space in the absence of these mechanisms. T-lymphocytes may also play a role in the delineation of viral spread (37). Factors regulating these mechanisms

have yet to be elucidated, but most likely involve immune-mediated cytokine production and the induction of major histocompatibility antigen expression within the nervous system.

5. GENE THERAPY

5.1 Neuronal vectors

The advances of modern molecular biology and *in vivo* gene therapy have challenged neuroscientists with the potential prospect of gene manipulation in postmitotic neurons. The ability to alter gene expression in these cells would open the door towards potential therapies for several disorders such as Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis. Gene therapy using viral-based vectors has received considerable attention and represents a major focus of ongoing research in many laboratories. Viral vectors using several different human viruses such as adenoviruses, retroviruses and herpes viruses are currently being developed. Gene therapy directed towards neuronal cells however, presents unique problems. These problems include the genetic manipulation of post-mitotic (*i.e.*, non-dividing) cells, the ability to specifically infect neurons, long-term maintenance of the vector DNA and expression of the target gene within the neuronal cells. Herpesviruses, particularly herpes simplex virus type 1, have unique characteristics of infection, replication and pathogenesis which make them potentially ideal candidates for the development of viral vectors capable of altering endogenous gene expression or delivery of foreign genes both *in vivo* and *in vitro*. The reader is directed to several reviews on these subjects (38-41).

5.2 HSV-based vectors

Herpes viruses have several advantages which lend to their ability to act as neuronal vectors. The HSV genome has been sequenced in its entirety and is rather extensively studied (9). As a result of many years of intense research, a general knowledge exists of which genes and DNA sequences may be deleted and at which sites foreign DNA may be inserted into the DNA genome (10). These studies also have defined the minimal requirements for viral replication and packaging (41). HSV-based vector strategies rely on the ability of HSV to infect neuronal cells and to establish a latent infection. Latency is defined as a state in which viral DNA is maintained within the cell nucleus in the absence of any viral replication. During latency, viral gene expression is largely absent with the exception of the latency-associated transcripts (LAT's) which may remain transcriptionally active (8).

The two main strategies for HSV-based vectors in use today are genetically-engineered viruses and plasmid derived "amplicon" vectors. The first strategy involves the construction of recombinant viruses containing deletions in one or more viral genes whose expression is essential for viral replication (for reviews,

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see 38-39). These viruses are incapable of producing a productive viral infection (*i.e.*, they are replication incompetent) in normal cells and require a complementing cell line (a cell line that can supply the deleted protein(s) to the virus in *trans*) for replication. Foreign genes can be inserted into these mutated viral genomes with the goal of producing a virus vector that will infect the target cell (*i.e.*, neurons), and express the foreign gene without killing the cell (due to viral replication). The second strategy involves the use of plasmid derived vectors containing HSV-1 origins of DNA replication and DNA packaging signals which enable multiple copies of the vector genomes to be packaged into helper virus virions (for reviews see 40, 41). Helper viruses can be either recombinant viruses containing a deletion within an essential viral gene or viruses containing temperature-sensitive mutations that prevent replication at 37° C (normal body temperature). In the case of the former, the replication of the helper virus and packaging of the amplicon vector DNA must occur in a cell line capable of complementing the mutations in the helper virus. Plasmid-derived vectors (amplicons) are advantageous because the DNA constructs can be easily manipulated to test endogenous, foreign, antisense or promoter gene expression in the target cell. Although the efficiency of delivery of these multiple copy vectors is high, the primary disadvantage of this system is the fluctuating helper virus to amplicon ratios with passage, which may result in some infected cells not receiving the amplicon genome. Viral titers must be monitored to ensure high amplicon delivery and experimental reproducibility in the absence of wild-type recombinants (41).

Regardless of the vector system used, two primary goals must be achieved to enable long-term gene expression in neuronal cells. The first goal involves the construct of mutant vectors which themselves are noncytotoxic to cells. Several studies have noted active expression of a foreign gene by HSV vector constructs which subsequently became inactivated (41-43). Reasons for this are not completely apparent, but evidence suggests that the inactivation is a result of cytotoxic effects induced by vector systems.

The second goal involves designing stable, active promoters capable of expressing appropriate levels of the foreign protein. The specific promoter involved in individual therapies may change according to the type, status and activity of the neuronal cell of interest. Originally, strong promoter systems such as the human cytomegalovirus IE promoter, the SV40 enhancer, and the RSV LTR were used to drive gene expression. Although such promoter systems were capable of expression they were only active transiently (1 week) and did not result in long-term gene expression (38, 39). Neuronal specific promoters (such as the neurofilament and neuronal-specific enolase promoters) which are believed to be constitutively active in neurons, also

produced only transient expression in several HSV vector constructs (38, 39).

During HSV latency the only viral transcripts consistently detected are the latency associated transcripts (LATs). The possibility that the LATs are constitutively expressed in latently infected neurons has made them strong candidates for long-term gene expression in neuronal systems. This hypothesis along with the goal of understanding possible functions and implications of the latent transcripts, has led to a vast literature focused on understanding LAT transcription. The identification of transcriptional activators and suppression mechanisms which may determine functionality in any promoter system is a difficult task considering the modulation which occurs in specific cell types and culture systems. Recently, it has been shown that plasmid derived vectors utilizing HSV-1 promoters are resistant to short-term inactivation and capable of long-term gene expression (44). One possible explanation could be the high copy number of amplicon molecules delivered to individual cells (45). Another explanation stems from data suggesting low level IE gene activity during latency (46). Regardless of the process of sustained activity IE promoters may serve as useful promoter systems in experimental gene transfer vectors.

6. SUMMARY

Herpes simplex virus, as a result of its rather unique life cycle in humans, is a useful tool for many areas of research. Its use in the field of neuroscience represents the newest and certainly one of the most interesting and complex directions of research. Studies utilizing HSV in gene therapy, neuronal tracings, and demyelinating diseases will continue for many years and should provide important insights in areas that up to now have been most difficult to study.

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