

# Vector-mediated Gene Therapy and the Herpes Simplex Virus

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The entirety of any organism is achieved primarily by the regulated expression of the components of its genome. We are the phenotypic result of our genotypic expression. That is, our gene products are integrally responsible for the physical, intellectual and behavioral traits that identify us as individuals. Genes are hereditary material composed of nucleotide sequences that code for the synthesis of proteins that are either structurally or metabolically active in living tissues. The tight regulation of gene expression is innately programmed and responsible for the intricately detailed assembly and function of the biological systems that make up an organism.

When the balance of our gene expression is distorted by outside influences or internal mishaps, the resulting malaise is commonly known as disease. Such alterations of the gene sequence or of the regulation of gene expression may result in protein products that either do not function properly or are not manufactured at all. The mutated or absent proteins may upset the normal activity of the cells and lead to the expression of an illness. Scientists have thoroughly dissected many diseases in attempts to develop therapies that either compensate for defective gene expression or that interfere with disease mechanisms. Current treatments often consists of oral drug administration to deliver temporary symptom relief. Many obstacles such as harmful side effects, drug resistance, cost and addiction associated with long-term use often accompany such therapy. In the search for more efficient and permanent alternatives, scientists are studying the potential use of viral vectors as a vehicle for gene therapy.

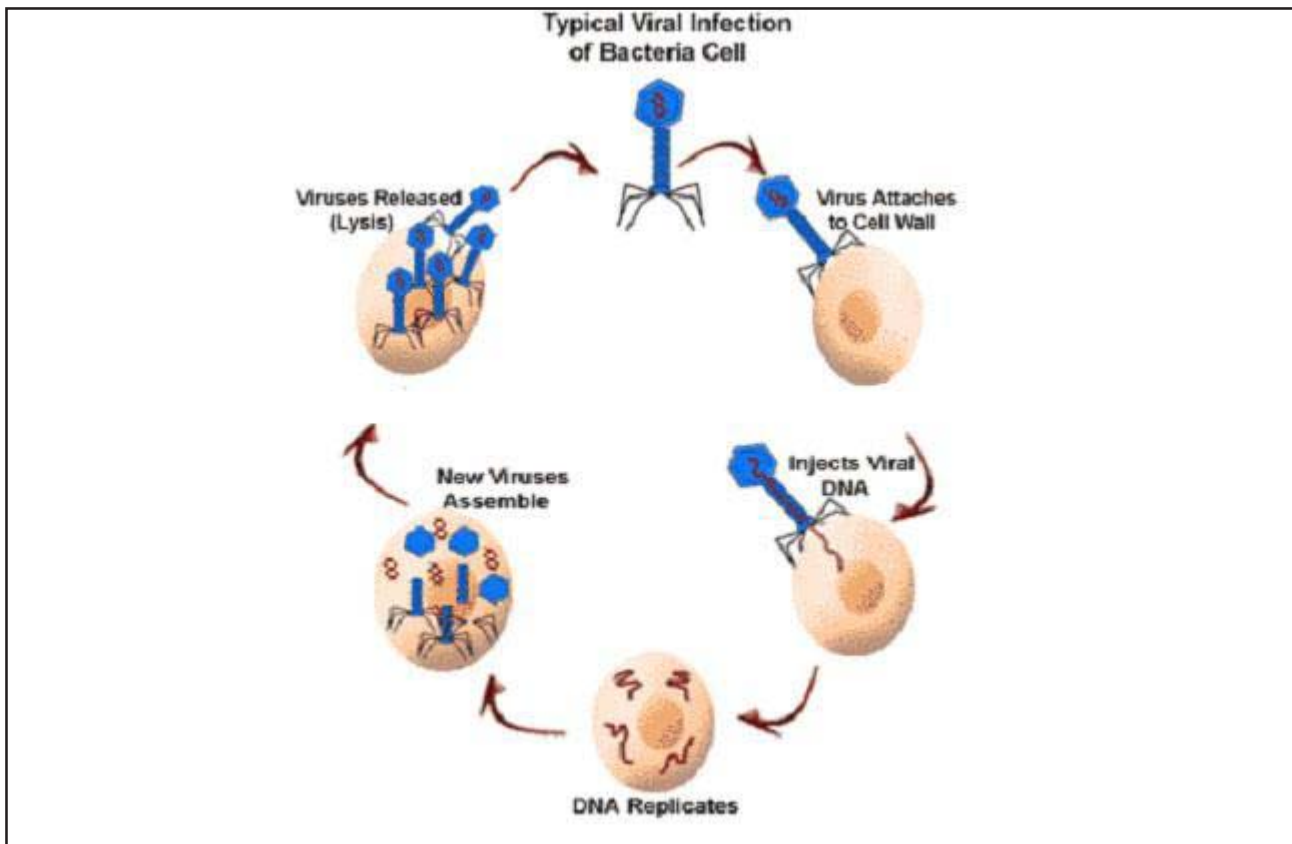
## Gene therapy

Gene therapy, in its broadest sense, refers to replacing or repairing a defective gene in the diseased

cell's genome in order to restore normal cell function and tissue integrity. Major obstacles medical scientists currently face include physically getting the new genomic material into the target cell and regulating its activity once it is there. Intense study of the infectious mechanisms of viruses has divulged their potential to act as vehicles for safely integrating recombinant genetic material into the diseased host cell's genome over the course of the natural viral life cycle. In nature, viruses rely on host cell machinery to replicate and to express the viral genome along with the cellular DNA in order to reproduce and infect other cells. Consequently, viral genetic material is combined with the cellular genome. The use of an attenuated viral vector for gene therapy would enable the incorporation of a compensatory gene into the genome of a diseased cell. The expression of that gene would correct for the manifestation of the disease in the patient.

The making of a viral vector requires sufficient knowledge and an effective strategy. First, the viral life cycle and its genetic sequence must be known in order to splice out the non-essential and virulence genes. This genome deletion enables the construction of a non-pathogenic vector, which is unable to harm the patient. The virus is genetically modified to retain its infective ability but not its replicative ability. This prevents the spread of the recombinant gene to healthy cells and ensures a single round of transfection. The latter introduces the problem of transient expression in non-dividing cells. Such cells are characterized by declined gene expression. Intermittent transfections with the vector would likely be required to achieve the long-term desired effects. The recombinant gene is inserted with adequate viral and cellular promoters present to ensure its recognition by the transcriptional machinery.

The threat of a harmful immune response should be considered a barrier for the use of viral vectors.



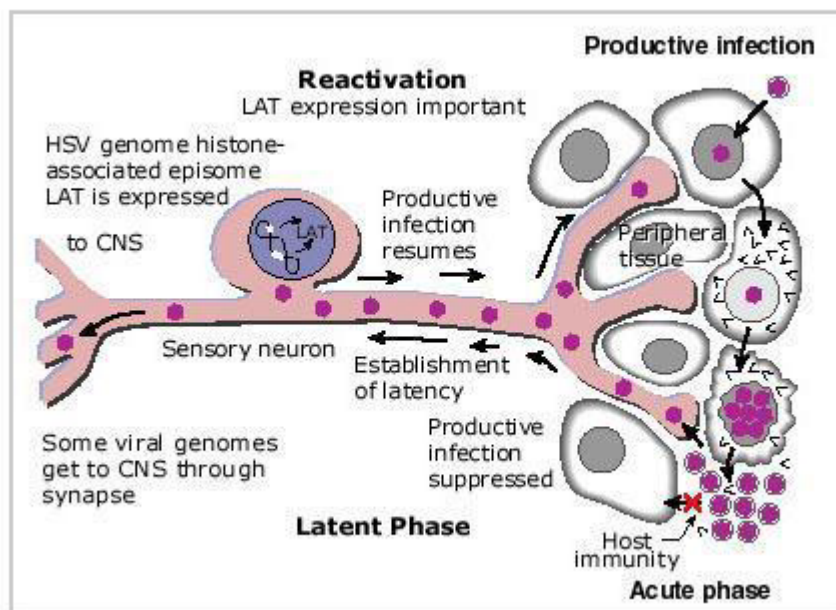
**Figure 1.** The general life cycle of a virus.

The body's response to the presence of foreign material may result in severe tissue damage. Consequently, addressing the disorder with genetic manipulation may introduce an equally significant immune reaction problem. Furthermore, viral genes are composed of fewer nucleotides than human genes. Therefore, a virus big enough to accommodate the recombinant genetic material is another prerequisite to vector therapy. As compared to somatic peripheral conditions, neurological diseases have the unique obstacle of the blood brain barrier preventing entry of the vector into the central nervous system (CNS). The herpes simplex virus' (HSV) natural neurotropism has presented it as the most suitable candidate for targeting neurological disorders. In fact, HSV type I has been shown to overcome all of the above-mentioned obstructions and is considered a well-suited vector for the treatment of CNS disorders.

The first human herpes virus to be recognized, HSV, is one of the most extensively studied viruses and one of the most abhorred pathogens for the general population. The three ubiquitous types of HSV in the

alphaherpesvirinae subfamily of human herpes viruses, HSV-I, HSV-II and HSV-III, are transmitted via close contact and are known for causing lytic infections of fibroblasts and epithelial cells, persistent infections of lymphocytes and macrophages and latent infections in neurons<sup>1</sup>. An infection with HSV-I results most commonly in cold sores. Infection by HSV-II is responsible for the reputable genital lesions. HSV-III, varicella-zoster virus, is most renowned for producing chicken pox. Normal infection leads to major structural and biochemical alterations that ultimately result in cell destruction.<sup>2</sup>

Herpes simplex begins its life cycle by binding heparan sulfate, a proteoglycan found on the surface of many cell types. It subsequently interacts with one of several cellular receptors closer to the cell surface and fusion with the cell membrane occurs.<sup>1</sup> Once inside the cell, the virus travels along the host cytoskeleton to the nucleus. There it interacts with the host transcriptional machinery enabling replication, assembly and budding from the nuclear membrane.<sup>3</sup> HSV's ability to infect the brain may enable it to be used as a



**Figure 2.** Lytic and latent infections by herpes simplex virus<sup>15</sup>.

vehicle for gene transport to the CNS. In traditional drug delivery systems, the blood brain barrier presents a physical obstacle preventing the target from being reached whereas HSV has a natural tendency to infect neurons. HSV begins by replicating at the base of the lesion in epithelial cells. It then spreads to peripheral nerve endings associated with the epithelium, travels along the axon by retrograde axonal transport facilitated by cellular microtubules, and enters the nucleus where it assumes a latent state without damaging the neuron<sup>2,4</sup>. The high levels of viral replication at the peripheral site of infection initiate syncytia formation which facilitates the cell-to-cell spread<sup>1</sup>. Reactivation of the herpes virus genome from latency is triggered by physical or emotional stress, peripheral tissue damage, or epinephrine and prostaglandin release. This re-awakening is coupled with axonal transport of the new virus progeny to the site of entry in the peripheral tissues where symptoms recur<sup>2</sup>. The maintenance of latency is a passive event. The only viral gene expression present involves that of a single latency-associated transcript (LAT), which is encoded with a neuron-specific weak promoter. The LAT gene products accumulate in the nucleus and are not required for viral survival<sup>5</sup>. Foreign gene insertions in the HSV LAT-gene region would promote restricted expression in neurons. HSV can remain in this latent

state for years. Consequently, the potential for sustained expression of the therapeutic gene inserted in the LAT region is significant would be important to therapies involving post-mitotic cells such as CNS neurons.

Herpes Simplex virus is an enveloped, icosahedral and double stranded DNA virus large enough to encode roughly 70 transcripts of which only half are required for viral replication<sup>6</sup>. This suggests ample space for recombinant gene insertions and the delivery of multiple genes in one vector. Like other large DNA viruses, HSV has also evolved a variety of mechanisms to evade the host immune responses to infection. The Fc and complement receptors on the surface of HSV weaken humoral responses while its cell to cell spread and latency in neurons facilitate the evasion of antibody neutralization and clearance<sup>1</sup>. HSV is also capable of inhibiting antigen presentation to CD8 cells by blocking the viral peptide presentation by the MHC-I of the infected cells<sup>2</sup>. HSV possesses a number of valuable characteristics such as natural neurotropism, a high transduction efficiency, the ability to infect post-mitotic cells, a large transgenic capacity, and the unique ability of assuming a latent state in neurons. These factors present HSV as a prime candidate for use in vector-mediated gene therapy of disorders involving the CNS. As HSV has addressed

and conquered most of the barriers to vector therapy, the choice of recombinant gene to be inserted into the vector proves to afford limitless opportunity in the treatment of diseases.

A number of strategies for gene therapy in the central nervous system have been explored:<sup>1</sup>

### ***Replacement of missing or defective genes***

In the event of an inherited single gene disorder (such as Lesch-Nyhan syndrome) where the complete DNA sequence, cause, and effect of the disorder are known, a single gene replacement is appropriate.

### ***Enhancement of growth factor or enzyme production***

In the vicinity of CNS lesions, where the pathology and pathogenesis are understood, this approach has demonstrated symptomatic relief despite inadequate understanding of the genetic basis of disease.

### ***Virus-directed enzyme pro-drug therapy***

The delivery of a drug-sensitivity gene would be beneficial in the treatment of a malignant brain tumour making it more susceptible to conventional anti-cancer agents.

### ***Direct cell killing***

Using the inherent ability of some viruses to replicate in, and directly kill, rapidly growing cells, such as tumour cells, while not affecting normal cells could be used in conjunction with the delivery of a drug-sensitivity gene for optimal destruction of a tumour.

### ***Vector-mediated delivery of anti-sense oligodeoxynucleotides (ODNs)***

The ODNs, short segments of DNA, would bind to complementary mRNA and block the expression of specific genes within cells. The encoded protein would fail to be synthesized, as the mRNA would not be recognized by the translational components of the

cell. In this case, a harmful gene product would be targeted.

### **Insertion of major histocompatibility complex (MHC) genes**

An increase in cellular antigen expression of tumour cells would enhance the immune response and increase the susceptibility of such cells to host cytotoxic immunity.

### **Conclusion**

It has been demonstrated that the use of viruses in vector-mediated gene therapy, in particular those derived from herpes simplex virus, has the potential to become a significant therapeutic option for a variety of neurological diseases. HSV vectors support the inclusion of large cellular promoters that can direct cell-type specific gene expression. They have the capacity for retrograde transport along neuronal axons to the central nervous system. They warrant regulation of molecular mechanisms producing disease, and they provide the opportunity to evaluate other neuroprotective genes whose actions may be cellularly autonomous.

Although there remain technical and ethical problems to overcome, further applications of viral vectors are continually being discovered. An increasing number of previously incurable diseases now have the potential to be conquered. Nonetheless, there is still a need for further development and optimization of gene therapies in order to face the onset of new diseases and to fully conquer old ones. As medical science continues to press forward, the world stands back in awe of the unimaginable idea that something with the stigma of a virus can be disabled and manipulated into a vehicle for gene therapy.

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