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## Invited Review

# Gene therapy using herpes simplex virus-based vectors

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**Summary.** Gene therapy involves the use of specific genes to treat human diseases and is thus critically dependent on efficient gene delivery systems. Although a variety of systems for such gene delivery are under development, HSV has unique advantages in terms of its large genome size and for gene delivery in the nervous system because of its ability to enter a latent state in neuronal cells. Considerable progress has been made in the effective disablement of this virus whilst retaining its ability to deliver genes and in producing long term expression of the foreign gene. Although much remains to be achieved in the further disablement of the virus and its testing in rodent and primate models of human diseases, it is likely that these viruses may ultimately be of use in human gene therapy procedures particularly for otherwise intractable neurological diseases.

**Key words:** Gene therapy, Herpes simplex virus, Virus vectors, Neurological diseases

### Introduction

Gene Therapy is used to describe the delivery of a gene or genes to an individual suffering from a specific disease with the aim of achieving a therapeutic benefit (for an earlier review see Martin Duque et al., 1998). The most obvious examples of this will be where an individual is suffering from the effects of a genetic disease caused by a mutation in a specific gene. In such cases, delivery of a functional copy of that gene could provide effective relief of the disease. As well as being applicable to such genetic diseases, the gene therapy approach is also applicable to situations other than those where the primary genetic defect is understood or which do not have a genetic component. Thus, for example, gene therapy for cancer can involve the delivery of genes which stimulate an immune response to the tumour cells or which allow a non toxic pro-drug to be converted to a toxic form by the cancer cell, thereby killing the cell. Hence gene therapy can involve the delivery of genes which are of therapeutic benefit in the disease regardless of whether the disease has a genetic component or whether such a genetic component is fully understood.

This second type of gene therapy is particularly applicable in diseases of the nervous system where many of the complex diseases either do not have a simple genetic basis or that basis is not understood (for review see Latchman, 1996). Thus, in neurodegenerative diseases such as motor neuron disease or Alzheimer's disease one could supply the gene encoding a neurotrophic factor which could promote survival of neurons that would otherwise die. Indeed, therapeutic benefit has been observed in an animal model of motor neuron disease when the genes encoding neurotrophin-3 and ciliary neurotrophic factor were delivered with virus vectors (Haase et al., 1997).

Similarly, it has been shown that the symptoms of Parkinson's disease can be relieved both in animal models and human individuals by the transplantation of foetal neurons, because such neurons provide a source of dopamine to replace the deficit of this substance caused by the loss of dopaminergic neurons during the course of the disease (Backlund et al., 1985). Hence, gene therapy for Parkinson's disease could be attempted by delivering the gene encoding tyrosine hydroxylase which is the rate limiting enzyme for dopamine production. Similarly, it would be possible to deliver the gene encoding glial derived neurotrophic (GDNF) factor since this factor has been shown to promote the survival of dopaminergic neurons and hence could be used to arrest the progressive loss of dopaminergic neurons which is characteristic of the disease (Opacka-Juffry et al., 1995; Lapchak, 1996; Lapchak et al., 1996).

### Methods of gene delivery

These possibilities, have led numerous laboratories to attempt to develop means of effectively delivering genes *in vivo*. One means of doing this would be to transplant cells engineered to express the gene of interest. Thus, fetal neuronal transplants can be beneficial in neurological diseases (see above) but the material is scarce and ethical difficulties arise. A possible solution would be to engineer non-neuronal dividing cells such as fibroblasts to produce the

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appropriate factors and then transplant these cells into the brain. In this situation, the gene can be delivered to the dividing cells by retroviral vectors that would not serve the purpose in neurons since they do not infect non-dividing cells (Miller et al., 1990). Thus, use of fibroblasts or myoblasts engineered to produce tyrosine hydroxylase has successfully lessened rotational behaviour in the 6-hydroxydopamine rat model of Parkinson's disease (Fischer et al., 1991). Drawbacks of this technique include the potential for tumorigenesis when dividing tumour cell lines are used and a rapid decrease in expression of the transgene when primary fibroblasts are used (Senut et al., 1996).

As well as such *ex vivo* cell transplant approaches one could envisage delivering genes directly to any organ including the brain The simplest means of doing this would be the direct injection of DNA in a standard plasmid vector. However, such methods have proved to be of relatively low efficiency for organs other than skeletal muscle. Indeed, even when the DNA is coated with cationic liposomes to enhance gene delivery, only relatively low levels of gene expression are obtained following injection into the brain for example, (Tsuda and Imaokea, 1996).

Thus, many laboratories have turned their attention to virus vectors since viruses naturally deliver their genetic material into the cells of the organisms which they infect. Although most retroviruses will not infect non-dividing cells (Miller et al., 1990) and therefore cannot be used for direct gene delivery to the brain, lentiviral vectors based on the human immunodeficiency virus (HIV) type of retrovirus will infect non-dividing cells. Vectors based on lentiviruses and other viruses such as adenovirus, adeno-associated virus and herpes simplex virus have all been used as gene delivery vectors capable of delivering genes to non dividing cells in the brain.

## Advantages of herpes simplex virus as a vector

In comparing such viral vectors, in terms of their genome size (Table 1) it is immediately apparent that HSV has a much larger genome than any of the other viruses. This is of particular importance since a virus will only package the appropriate size of nucleic acid into a virus particle. Hence, viruses with small genome sizes cannot accept very large DNA inserts even when the majority of their genes are eliminated. This is of particular significance when it is necessary to introduce a large gene into cells or when effective therapy may require the delivery of more than one gene. Thus, for example, when adenovirus vectors were used in an animal model of motor neuron disease, effective therapy required both neurotrophin-3 and ciliary neurotrophic factor which had to be delivered in two separate adenovirus vectors (Haase et al., 1997). This would not have been necessary with HSV due to its much larger genome size. Similarly, to produce an optimal effect in Parkinson's disease using the gene encoding tyrosine hydroxylase, it will probably be necessary to deliver also the gene encoding GTP-cyclohydrolase 1 which catalyses the rate limiting step in the pathway that synthesises the tyrosine hydroxylase co-factor, tetrahydrobiopterin (Bencsjcs et al., 1996).

In addition, whilst all the viruses can infect neuronal cells, only HSV naturally infects neuronal cells as part of its normal life cycle. Indeed, following initial infection at the periphery the virus migrates up the sensory nerve processes innervating the site of initial infection and establishes life long latent infections of the neuronal cell bodies without in any way damaging the neuron or compromising neurological function (for review see Roizman and Sears, 1987; Latchman, 1990). Such latent infections serve as a reservoir for further infections at the periphery following reactivation of the infection but such reactivation once again does not apparently damage the neuronal cell.

## Disablement of the HSV vector

Hence, this ability to deliver its DNA to neuronal cells without causing any damage renders HSV ideal for similarly delivering a foreign gene which it is necessary to express within the neuronal cell. Of course, in order to achieve this it will be necessary to disable the virus so that it can no longer conduct the lytic replication which produces peripheral facial and genital sores. This is particularly true since direct injection of virus into the brain results in lytic replication leading to encephalitis and rapid death. Therefore if advantage is to be taken of the ability of the virus to enter a latent state in neuronal cells, the potentially damaging effects of lytic replication must first be eliminated.

Evidently, it is essential to achieve this disablement in a manner which still allows the virus to be grown up so that stocks can be prepared for *in vivo* injection. It is this requirement to disable the virus for use *in vivo* whilst still allowing stocks to be prepared *in vitro* which constitutes the essential problem in effectively disabling the virus. Two basic methods of achieving this have been proposed and these will be discussed in turn.

The first method involves the inactivation of a virus gene which is essential for lytic replication in all cell types. The most frequently inactivated gene in vectors of this type is that encoding the essential immediate early protein ICP4 (Chiocca et al., 1990; Dobson et al., 1990). It is also possible to inactivate the other essential immediate early gene which encodes the viral ICP27 protein (Sacks et al., 1985). Removal of either of these genes effectively renders the virus incapable of lytic replication in any cell type and it will not therefore

**Table 1.** Genome size of potential virus vectors for the nervous system

| | |
|---|---|
| Adeno-associated virus | 8,500 base pairs |
| Adenovirus | 35,000 base pairs |
| Herpes simplex virus | 150,000 base pairs |
| Lentivirus | 10,000 base pairs |

produce encephalitis when injected into the brain.

The problem evidently remains however, of growing these viruses in order to produce sufficient stocks. This is achieved by growing the virus on cell lines which have been artificially engineered by introduction of the gene encoding ICP4 or ICP27 and therefore allow lytic growth of the virus. Although this procedure does allow the virus stocks to be grown up, it suffers from the disadvantage that recombination can occur between the defective virus genome lacking functional ICP4 or ICP27 and the functional ICP4 or ICP27 gene in the cell line. This will evidently result in the regeneration of dangerous wild type virus.

To overcome this problem, an alternative approach is to inactivate a virus gene which is necessary for replication in non-dividing cells such as neurones but is not required for replication in dividing cell types. Hence, the virus will not produce encephalitis since it will not replicate lytically in the brain but stocks can readily be grown on dividing cells *in vitro*. Because of the advantages of this method, our laboratory has utilised a virus strain lacking the gene encoding ICP34.5. The removal of this gene renders the virus non neurovirulent upon injection into the brain but continues to allow it to replicate on dividing cells (Maclean et al., 1991). We have introduced a marker ß-galactosidase into this vector and shown that it is able to deliver genes to the central and peripheral nervous system following direct injection into the brain or the footpad of mice respectively (Coffin et al., 1996a).

This system thus combines the ability of the virus to be grown up in culture without a complimenting virus gene expressed in a cell line, with the lack of damaging encephalitis upon injection into the brain allowing gene transfer without damaging side effects. Unfortunately however, in these experiments only a relatively small number of cells in the brain successfully expressed the marker ß-galactosidase following injection (Coffin et al., 1996a). We therefore developed this vector further by inactivating also the gene encoding the viral ICP27 protein thereby introducing two defects into the virus genome. Very surprisingly, the resulting virus not only produced considerably less damage when injected into the brain *in vivo* but it was also capable of a much higher efficiency of gene delivery with approximately one thousand fold more neurons expressing the marker gene than with the single mutant virus lacking only functional ICP34.5 (Howard et al., 1998). This double mutant virus therefore has both added safety since it contains two disabling mutations but is also a much higher efficiency gene delivery vector.

This virus does however, have to be grown on a cell line which artificially expresses ICP27 since this protein is essential for lytic growth of the virus on all cell types (Sacks et al., 1985). The possibility of recombination resulting in a functional ICP27 gene being regenerated within the virus is minimised however by completely deleting the ICP27 gene from the virus and ensuring that there is no overlap between the DNA sequences remaining in the virus genome and those contained within the cell line. Moreover, even if a rare recombination event were to occur, this would still not regenerate a wild type virus since the resulting virus would continue to lack ICP34.5 and would still not cause a damaging encephalitis.

Hence this doubly disabled virus represents a relatively safe and efficient gene delivery vector which can be used as a platform for further disablement. This has now been achieved by deleting the gene encoding the other essential immediate early protein ICP4 and inactivating the viron transactivator protein Vmw65 (Thomas et al., 1999). This virus thus has four defects preventing its lytic growth in the brain and may therefore ultimately be safe enough for therapeutic use in humans.

### *Use of HSV as a vector*

Once the virus has been effectively disabled, it is possible to use it as a gene delivery vehicle. One approach to this utilised by our laboratory will be discussed here and involves the introduction of the exogenous gene into the virus by recombination. An alternative approach using so called 'amplicon' vectors relies on introducing the gene of interest into a plasmid which contains an HSV origin of replication and packaging signal. This construct is introduced into cells by transfection and the cells are then infected with a helper HSV virus so that the amplicon becomes packaged into viral particles. This system is discussed extensively elsewhere (Leib and Olivo, 1993; Latchman, 1994; Coffin and Latchman, 1996; Fink et al., 1996, Kennedy, 1997).

In the recombination method the foreign gene of interest together with an appropriate promoter is flanked by two adjacent regions of the virus genome in a plasmid vector. If this vector is then introduced into cells by transfection together with full length viral DNA, recombination will occur between the viral DNA in the plasmid and the equivalent sequences in the virus, resulting in the insertion of the gene into the virus (Fig.1). This method was first used to introduce marker genes such as the ß-galactosidase gene (Ho and Mocarski, 1988) or the hypoxanthine phosphoryibosyl-transferase gene (Palella et al., 1988) into the virus genome and viruses constructed in this manner have been shown to effectively deliver such genes to the central and peripheral nervous systems *in vivo* (Dobson et al., 1990; Bloom et al., 1995).

Following recombination, some virus particles will contain recombinant virus having the gene of interest whilst others will contain the original non-recombinant virus. However, it is relatively easy to separate these two types of viruses. Thus, both the recombinant and non recombinant viruses will form plaques on suitable cell lines which compliment any defects in the vector and the recombinant virus can therefore be purified provided it can be distinguished from the non recombinant virus. This can be achieved, for example, by introducing the

gene of interest into the thymidine kinase locus of the virus thereby inactivating this locus and rendering the resulting virus resistant to the effects of the anti-herpes drug acyclovir (Ho and Mocarski, 1988; Palella et al., 1988). Alternatively, the recombinant virus can be distinguished on the basis that it contains a readily assayable marker gene such as ß-galactosidase or green fluorescent protein (GFP) either alone or in association with the gene of interest. If this cannot be achieved it is also possible in all cases to grow virus from individual plaques and identify those carrying the gene of interest by southern blot hybridisation.

## Long term gene expression in HSV vectors

However, before such viruses can be used effectively for gene therapy, it will be necessary to overcome another obstacle. Thus, many of the applications of gene therapy for example in the nervous system such as the treatment of Parkinson's disease will require long term expression of the therapeutic gene. Unfortunately however, during the onset of latent infection the virus shuts down the expression of virtually all of its own genes in order to enter a virtually silent latent state (for review see Roizman and Sears, 1987; Latchman, 1990). This shut down of gene expression also occurs for any exogenous genes which are introduced into the virus genome either under the control of the viruses own promoters, other virus promoters such as the immediate early promoter of cytomegalovirus or a variety of cellular promoters (Palella et al., 1988; Ho and Mocarski, 1989; Fink et al., 1992; Lokensgard et al., 1994). This results in expression of the foreign gene being observed for only a few days at the most.

Clearly, some means must be found of overcoming this problem if HSV vectors are to be used successfully in a variety of diseases where long term expression of the therapeutic gene will be required. One approach to this problem, involves taking advantage of the fact that a small region of the HSV genome remains active during latency producing the so called latency associated transcripts (LATs) (Croen et al., 1987; Stevens et al., 1987). (Fig. 2). The expression of these LAT transcripts is driven by two adjacent promoters known as LATP1 and LATP2 and a number of laboratories have attempted to use these to drive long term expression of a foreign gene. This has been achieved either by inserting the foreign gene into the LAT region hence placing it under the control of the LAT promoters or by introducing the foreign gene linked to LATP1 or P2 into another region of the genome.

In general, experiments with LATP1 have indicated that it is not sufficient for long term expression either when a gene is inserted directly downstream of it into the LAT locus or when a LATP1 promoter linked to the ß-galactosidase gene is inserted into another site in the HSV genome (Margolis et al., 1992, 1993; Dobson et al., 1995). In contrast the LATP2 promoter does appear to give much longer term expression either within the LAT region or elsewhere, although such expression is relatively weak (Ho and Mocarski, 1989; Goins et al., 1994).

To overcome these difficulties, our laboratory has explored the possibility of whether a region containing LATP2 (which we call LAP2) could act as an enhancer element conferring long term expression upon a strongly active promoter. Thus, we have linked LAP2 to the very strong cytomegalovirus immediate early promoter which normally gives only very short term expression within an

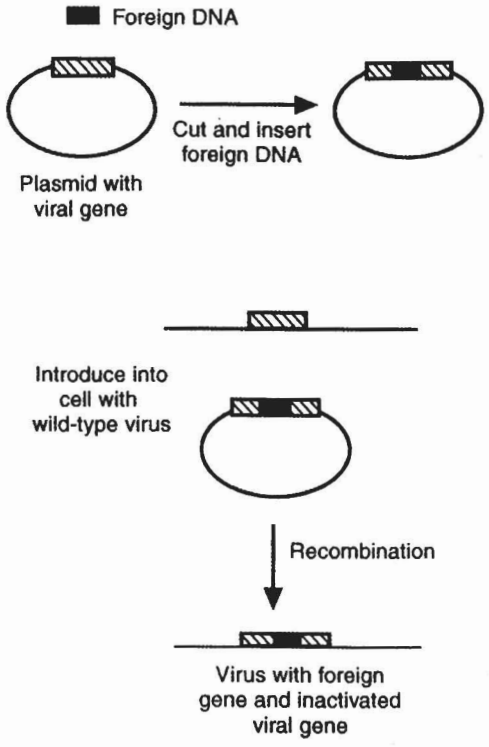


**Fig. 1.** Introduction of foreign DNA into the HSV-1 genome by recombination.

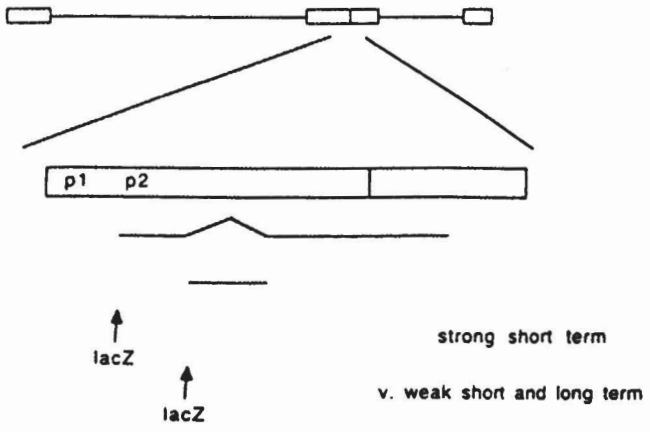


**Fig. 2.** Position of the Lat region (boxed) and the two promoters (p1 and p2) in the HSV genome. The two Lat RNA transcripts are illustrated. These are an 8 kilo-base RNA which is spliced (as indicated by the triangle) to produce the 2 kilo-base stable RNA. The effect of linking each of these promoters to a marker gene (Lac Z) is also shown.

HSV vector and shown that this combination can direct strong long term expression when introduced into both the central and peripheral nervous systems. This is likely to be achieved by the LAP2 region acting as a signal to maintain the viral chromatin in its immediate vicinity in an open configuration consistent with transcription thereby allowing the CMV IE promoter to produce high levels of transcription in the long term (Palmes et al., 2000).

We are currently exploring the possibility of also linking LAP2 to cellular promoters with cell-type specific patterns of activity in order to determine whether such cell type specific activity could be produced in the long term within a virus vector by use of the P2 element. The use of the P2 promoter has another advantage however, in that like classical enhancer elements it can act bi directionally and can therefore produce long term expression of two genes each under the control of their own promoters in opposite orientations with the P2 element contained at the centre of the construct. This is of particular importance where it is desired to produce long term expression of two genes and could be used, for example, where maximal therapeutic efficiency will require two genes to be successfully expressed.

In addition however, it could be used to express both a marker gene and a gene of therapeutic interest. This would, for example, facilitate purification of the virus away from non-recombinant virus (see above) on the basis of its expression of the marker gene with its subsequent use to express the therapeutic gene.

Another example of the use of such a virus containing both a marker gene and a therapeutic gene would be in situation where it is necessary *in vitro* to deliver a gene to a population of cells in culture with subsequent re-insertion of these cells into a patient. Thus, many cells for which it is desired to achieve this such as bone marrow stem cells or dendritic cells are extremely difficult to transduce with exogenous genes. In the system proposed, the virus expressing two genes would be used to infect such cells and the successfully infected cells could be sorted on the basis of their expression of a reporter gene producing a 100% pure population expressing the therapeutic gene for reintroduction into the patient.

We have shown the feasibility of this procedure by utilising a virus which expresses both green fluorescent protein (GFP) and ß-galactosidase and showing that sorting of the infected cells on the basis of their expression of GFP results in a 100% pure population of cells expressing ß-galactosidase (Coffin et al., 1998).

The progress made with disabled recombinant HSV vectors indicates that they represent an effective system for gene delivery. This is particularly so with the finding that such viruses do not recombine or reactive endogenous latent HSV upon introduction into the brain of latently infected animals (Wang et al., 1997). Similar results were obtained in a phase I clinical trial in which patients with glioma had intracranial injection of a virus lacking only ICP34.5, which is expected to replicate and kill the dividing glioma cells but not non-dividing neurons; although the great majority of the patients in this study had latent wild-type HSV, no reactivation of the wild-type virus was observed. No other damaging effects were noted in this trial despite the fact that, to achieve the effect of killing tumour cells, the virus has only a single disabling mutation which does not prevent replication in all cell types (Rampling et al., 1999). This indicates that such a reactivation of wild type virus already contained within the patient is unlikely to be a problem with highly disabled gene therapy vectors. Hence, the progress made with the disabling of the virus whilst retaining its efficiency in gene delivery and in obtaining long term expression offers hope that such viruses may be of ultimate use therapeutically.

## Applications of HSV vectors

In our laboratory we have already constructed viruses expressing both tyrosine hydroxylase and the neurotrophic factor GDNF. These viruses have been successfully used to express the appropriate genes and produce a reduction in rotational behaviour in the 6-hydroxydopamine model of Parkinson's disease (Latchman and Coffin, 2000). Similarly, we have utilised these viruses to express the genes encoding individual heat shock proteins. As with delivery of hsp genes by standard transfection of plasmid constructs (Amin et al., 1996; Wyatt et al., 1996) such over expression, for example, of the 70kDa heat shock protein (hsp70) can protect cultured neuronal cells from subsequent exposure to thermal stress or ischaemia. Moreover, over-expression of the 27kDa heat shock protein (hsp27) with an HSV vector protected the cells not only against thermal or ischaemic stress but also against stimuli which induce apoptosis which was not observed with hsp70 (Wagstaff et al., 1999). Most importantly, the protective effect observed in these experiments was significant not only compared to a virus expressing a marker gene alone but also to samples of cells which were not treated prior to exposure to the stressful stimuli. Hence, the virus does not produce significant damaging effects itself and can deliver a foreign gene with an appropriate protective effect.

In similar experiments, we also observed a protective effect when these viruses expressing heat shock proteins were used to infect cells of cardiac origin (Brar et al., 1999). This illustrates, that HSV can also be used to deliver genes to other cell types and organs. Thus, we have previously shown that the virus can effectively deliver a marker gene to the heart *in vivo* (Coffin et al., 1996b) and similar results have also been observed in skeletal muscle (Huard and Glorioso, 1995) and in liver cells (Lu et al., 1995). Moreover, the ability to deliver to dendritic cells (see above and Coffin et al., 1998) is of particular importance in view of the high efficiency of antigen presentation by these cells which could be of use in cancer therapy.

Hence, whilst this review has concentrated on gene

delivery to neuronal cells because of the particular applicability of HSV to this relatively difficult problem, the development of HSV vectors may also be of use for gene delivery to a variety of other tissues and cell types. Ultimately, therefore it is likely that safe and effective HSV-based vectors could be used in gene therapy procedures to treat patients suffering from neurological and other diseases.

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