Transduction of Brain by Herpes Simplex Virus Vectors

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An imposing obstacle to gene therapy is the inability to transduce all of the necessary cells in a target organ. This certainly applies to gene transfer to the brain, especially when one considers the challenges involved in scaling up transduction from animal models to use in the clinic. Non-neurotropic viral gene transfer vectors (*e.g.*, adenovirus, adeno-associated virus, and lentivirus) do not spread very far in the nervous system, and consequently these vectors transduce brain regions mostly near the injection site in adult animals. This indicates that numerous, well-spaced injections would be required to achieve widespread transduction in a large brain with these vectors. In contrast, herpes simplex virus type 1 (HSV-1) is a promising vector for widespread gene transfer to the brain owing to the innate ability of the virus to spread through the nervous system and form latent infections in neurons that last for the lifetime of the infected individual. In this review, we summarize the published literature of the transduction patterns produced by attenuated HSV-1 vectors in small animals as a function of the injection site, and discuss the implications of the distribution for widespread gene transfer to the large animal brain.

Received 19 December 2005; accepted 18 August 2006. doi:10.1038/sj.mt.6300018

CHALLENGES OF GENE TRANSFER TO THE BRAIN

A variety of diseases affect the central nervous system (CNS), ranging from genetic disorders to cancer. Gene therapy has the potential to treat brain disorders at the molecular level by introducing genes into cells and the subsequent production of a therapeutic protein. Direct gene transfer into the brain has been extensively studied because it is refractory to many systemic treatments.[1,2] This organ poses an imposing target for gene transfer for several reasons. Introduction of vectors into the bloodstream is generally an ineffective way to target them to brain tissue owing to the blood–brain barrier. Thus, most methods to introduce genes into the brain rely upon intracranial injection. The surgery required for brain gene therapy is a major procedure, especially for patients whose health status is compromised. When injected into adult brains, gene transfer vectors predominantly transduce the area near the injection site, with relatively limited spread to other regions in most studies.[3–14] More extended transduction has been noted in neonatal animals when the rodent brain is undergoing major turnover and remodeling,[15] after injection into cerebrospinal fluid,[7] when targeting transfer across the blood–brain barrier,[16] or when using alternate viral surface proteins.[17–20] The focus of this review is on injection of smaller volumes into the parenchyma of the adult brain so as to allow for a comparison of the various studies.

SOME DISEASES OF THE BRAIN REQUIRE WIDESPREAD GENE TRANSFER

Some brain diseases may be amenable to treatment by gene transfer to localized regions (*e.g.*, Parkinson's disease[21]), but most neurogenetic disorders affect widespread regions of the brain, such as the lysosomal storage diseases (LSDs).[22] In order to achieve an effective gene therapy treatment of a global brain disorder, the vector-delivered gene product must be targeted to many cells that are dispersed throughout the brain. A large number of brain injections would have to be performed in order to transduce the brain of a child, requiring extensive surgery. However, most gene therapy studies have shown relatively limited ability to target widespread brain regions for transduction in experimental animals when injected into the adult brain parenchyma.[3–14,19,20]

METHODS TO ACHIEVE WIDESPREAD TRANSDUCTION OF THE BRAIN

Various methods have been explored to increase the scope of transduction of the brain including the use of viral vectors and physical or chemical means, such as convection and disruption of the blood–brain barrier, respectively. Convection relies upon a pressure gradient to increase the distribution of agents through solid tissue and results in increased viral vector movement over diffusion alone.[23] Disruption of the blood–brain barrier may

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allow penetration of viral vectors into some areas of the brain, although this process cannot discriminate and may allow unwanted chemicals to enter the brain as well.[24]

VIRAL VECTORS FOR GENE THERAPY OF THE BRAIN

Many viruses have been manipulated to serve as gene transfer vectors. Examples of viral vectors used to accomplish gene transfer to the brain include adenovirus,[25] retrovirus (including lentivirus),[26] adeno-associated virus (AAV; a replication-defective parvovirus),[27] and herpes simplex virus (HSV).[28]

Adenovirus will infect cells of the brain, but early vectors were associated with toxicity in many experiments that led to an inability to achieve stable transgene expression over time. The advent of the "gutless" adenoviral vectors has improved the stability of transgene expression by eliminating the expression of viral proteins and thus diminishing the potential immune response.[29] Retroviruses of the oncoviridae family (*e.g.*, murine leukemia virus) do not infect post-mitotic cells and hence have limited utility in the brain.[30] However, lentiviruses are a subgroup of retroviruses that can infect post-mitotic cells and represent a promising viral vector for the brain.[26] Lentiviruses stably integrate into the host genome and have been shown to provide stable transgene expression. Further, infection can be targeted to specific cell types by substituting other envelope glycoproteins, which act as cellular ligands, a process referred to as pseudotyping.[31] AAV is also a promising vector because several AAV serotypes infect cells of the brain.[15,18–20] Various studies have indicated that AAV vectors are able to provide stable transgene expression.[27,32]

HSV-1 is the major viral vector known to naturally target neurons for infection. It is a promising vector for several reasons: HSV-1 has evolved to form a latent infection in neurons, characterized by a lack of detectable viral protein production but continued transcriptional activity; HSV-1 naturally spreads in the nervous system via neuronal transport; HSV-1 infects a variety of species (for preclinical evaluation of vectors in various animal models); HSV-1 has a large packaging capacity for multiple or large transgenes; and the HSV-1 genome remains episomal (thus avoiding a risk of insertional mutagenesis) but is stably maintained in neurons. Transduction by HSV vectors in the brain is generally more efficient than with the other vectors (lower titers are required), which may reflect the fact that neurons are naturally targeted for infection by HSV-1 and not the other vectors.

HSV IS A PROMISING VECTOR BECAUSE IT CAN SPREAD IN THE NERVOUS SYSTEM

HSV-1 is a neurotropic virus that is able to spread through the nervous system and establish a latent infection in neurons. The main mechanism for spread of HSV-1 in the nervous system is via neuronal transport, which is a part of the normal life cycle of the virus.[33] It should be noted that the non-neurotropic viral vectors, such as adenovirus, AAV, and lentivirus mostly transduce cells in the vicinity of the intraparenchymal injection site in the adult rodent brain,[3–15,34] although some examples of limited neuronal transport of these viruses have been reported.[17–19,35–42]

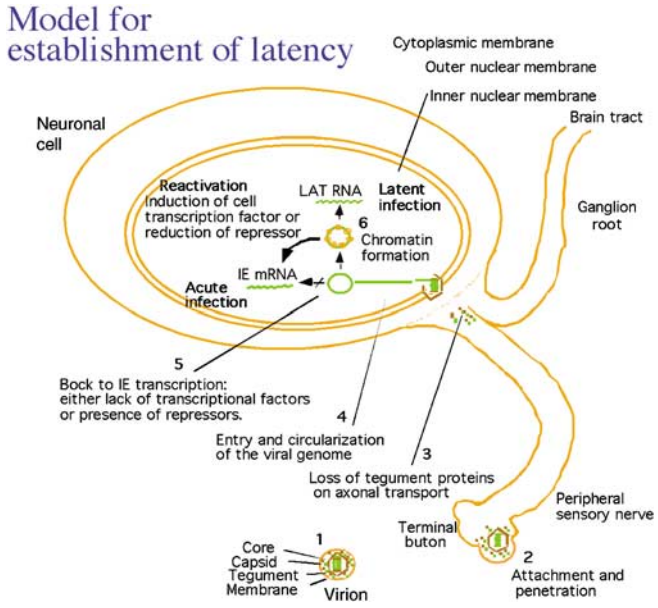

Figure 1 HSV-1 infects neurons and forms a latent infection in the nucleus. Following a lytic infection of the peripheral epithelium, HSV-1 enters sensory neurons and is trafficked by retrograde axonal transport to the sensory ganglia. A latent infection then ensues where gene expression from the viral genome is limited to the LATs. Although a PNS neuron is depicted, it is generally accepted that the events surrounding the formation of a latent infection in neurons of the PNS and the CNS (the focus of this paper) are similar. (1) The virus particle consists of a 150 kb double-stranded DNA genome within an icosahedral capsid. This capsid is surrounded by a tegument–lipid/protein material containing viral factors important for the initial stages of acute infection. The tegument is surrounded by a lipid membrane containing many viral glycoproteins that are important for attachment and penetration (2) of cell membranes. After penetration of the virion into the cell cytoplasm it has been seen in EM studies that the tegument can drift away from the capsids. (3) This might be expected to be most pronounced on the long journey from the peripheral axon of a neuron to the nucleus where the virion will dock with the nuclear pore and inject its DNA into the nucleus. (4) Upon insertion into the nucleus the viral DNA circularizes. (5) The insertion of DNA without the accompanying viral tegument proteins may render the initial genes to be transcribed (IE genes) inactive favoring the establishment of a latent infection. Alternatively a cellular protein (Zhangfei) may inhibit the ability of Vp16 to activate the IE genes.[44] With lack of gene expression or DNA replication the viral genome becomes progressively covered with nucleosomes and forms stable chromatin (6). Only one viral gene is expressed from this latent state (LAT). This gene is believed to be expressed for the life of the infected human. Long-term HSV vectors utilize this promoter by inserting a complementary DNA for the gene to be expressed in front of the LAT promoter.

During primary HSV-1 infection, virus replicates in epithelial cells near the site of initial exposure. The virus then enters sensory nerve endings and moves via retrograde axonal transport to the neuronal cell bodies of the sensory ganglia[43] where a latent infection can develop (**Figure 1**). Following the establishment of latency, viral reactivation can periodically occur, following which infectious virus can once again be detected. However, most gene therapy studies have shown relatively limited ability to target widespread brain regions for transduction in experimental animals when injected into the adult brain parenchyma.[3–14,18–20]

HSV-1 likely uses microtubule motors to perform neuronal transport as microtubule-depolymerizing drugs such as colchi-

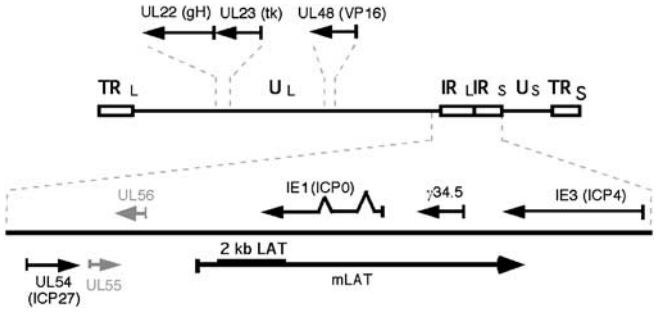

Figure 2 HSV-1 genes commonly deleted to create attenuated vectors. The entire HSV-1 genome is shown, with unique (U) and repeat (R) regions labeled. Terminal (T) and internal (I) repeats are also shown, as well as long (L) and short (S) regions/repeats. Genes in black are those deleted in the vectors, those in gray are not deleted and are included for reference. Deletions in the immediate-early genes ICP0, ICP4, and ICP27 prevent the initiation of the cascade of gene expression.[51] Deletions in the transactivator VP16 also prevent efficient expression of the immediate-early genes.[52] ICP34.5 is a neurovirulence factor and deletions in this gene increase the lethal dose by several orders of magnitude.[53] Thymidine kinase (tk) is necessary for the production of nucleotides in non-dividing cells.[54] gH is a glycoprotein with functions in entry, egress, and cell-to-cell spread of virus.[55,56]

cine, nocodazole, and vinblastine disrupt capsid transport.[45,46] The HSV-1 protein U_L34 is a component of the virion and is known to associate with both the major capsid protein ICP5 and the microtubule motor protein dynein. Thus, U_L34 may aid in tethering the virus capsid to the cellular machinery in order to perform neuronal transport. Indeed, disruption of the microtubular network by nocodazole treatment also results in a dispersal of U_L34.[47] Further consideration of the movement of HSV-1 in the retrograde and anterograde directions can be found elsewhere.[48]

Alpha-herpesviruses have been used as a sensitive agent for tracing neural pathways in the brain owing to their ability to be transported in neurons.[49] Most neuronal transport studies have been performed using lethal doses of pathogenic strains, followed by staining for viral antigens. Because wild-type HSV-1 causes encephalitis when introduced into the brain, gene transfer vectors based upon this virus must be modified in order to make them safe for brain injection. Three main strategies have been employed to accomplish this task: (1) deletion of genes that are dispensable for *in vitro* replication, but are essential for *in vivo* replication (replication-competent); (2) deletion of genes that are required for all forms of replication (replication-defective); and (3) deletion of all portions of the genome except for an origin of DNA replication and DNA packaging signal sequences (amplicons).[50,121] A map of the genes in which mutations have been introduced to make attenuated HSV-1 gene transfer strains is shown in **Figure 2**. A thorough discussion of all types of vectors and their production can be found elsewhere.[28,57,58]

These three types of HSV-1 vectors differ substantially in their similarity to wild-type HSV-1, which has been the model for defining a latent infection. Thus, it may not be appropriate to classify the infection carried out by attenuated vectors as *bona fide* latency. HSV-1 latency is defined as the ability of a virus to persist in a cell without expressing viral antigens, and to be able to reactivate to produce infectious virus at a later time. Most HSV-1 vectors are crippled in their ability to form latent infections by this strict definition. In fact, by this definition latency only occurs in the peripheral nervous system (PNS). Although molecular biology has shown that viral genomes do stably persist in the rodent CNS,[59,60] this is not latency from the virologist's point of view, because reactivation of the genome cannot be shown from CNS tissue.

POTENTIAL TOXICITY OF HSV-1

HSV-1 kills cells during the lytic stage of infection of an animal, including epithelial cells at the initial site of a natural infection and neuronal cells when it forms encephalitis. However, encephalitis is rare in humans. Most of the time the virus travels along neurons of the sensory ganglia and forms a latent infection in neuronal cell nuclei of the PNS. Nonetheless, HSV-1 DNA can commonly be detected in the post-mortem brain of apparently normal individuals (*i.e.*, no evidence of encephalitis) using sensitive techniques.[61,62]

However, when HSV is used as a vector for transmission of genes to the CNS it is crippled (as in the case of the ICP34.5 gene deletion) so that it cannot replicate in these critical cells. The clinical trials using HSV-1 ICP34.5 mutants indicated no encephalitis or other adverse effects,[63] whereas other reports have indicated severe inflammation in the rodent brain with the same strain.[64] Part of this discrepancy may be due to the purity of the vector preparation, which we have found to contribute to adverse affects following intracranial injection (unpublished observation).

In contrast, in research experiments where HSV-1 is used as a neuronal track tracer in animals, lethal doses/strains of virus have been used as these viruses give the clearest tracing data.[49] Thus, viruses with differing killing ability are used for different purposes and a number of viral mutations have been made to provide vectors with low to high toxicity in the infected animal and culture cell. For example, although HSV-1 ICP34.5 mutants can be grown to high titer in cultured cell lines, they are severely compromised *in vivo*.

SPREAD OF ATTENUATED HSV-1 GENE TRANSFER STRAINS

Much less is known about neuronal transport of attenuated HSV-1 gene transfer strains because the types of neural tracing studies described above were performed with wild-type virus, and have not been confirmed with attenuated strains. This is likely due to the fact that the goal of most gene transfer studies is to achieve maximal brain transduction, not to map neural pathways. As a result, larger volumes of virus are injected into the brain for gene transfer (1–5 μl) than can be used for sensitive tracing studies (0.1 μl). Movement of attenuated viruses has largely been studied by the expression of reporter genes[58,60] or by *in situ* hybridization of the latency-associated transcripts (LATs)[65,66] which are the only significant transcripts produced from the HSV-1 genome during latency.

A multitude of studies have shown that attenuated HSV-1 vectors are indeed trafficked to brain regions far from the injection site, similar to wild-type strains. This result has been achieved regardless of the type of HSV-1 vector used (replication

competent, replication defective, amplicon), the promoter used to drive transgene expression (LAT, exogenous, or other HSV-1 promoter), or the transgene itself.[51,60,64,65,67–79] Spread of attenuated HSV-1 vectors indicates that substantially fewer injections may be required to accomplish widespread gene transfer compared to other vectors. Many of these vectors were derived from strains that are unable to replicate in neurons *in vitro*. Thus, replication of attenuated HSV-1 in neurons is apparently unnecessary to achieve vector spread, although there is evidence that the titer of virus injection may dictate the extent of spread.[60] Spread of HSV-1 in neurons without a prerequisite for replication is not surprising in light of the normal life cycle of HSV, where replication in neurons cannot occur until after retrograde transport over a large distance to the neuronal nucleus. However, it is currently unknown if trans-synaptic spread (*i.e.*, infection of a secondary neuron) is possible with HSV-1 strains that are incapable of replication in neurons.

Although careful tract tracing has not been performed with attenuated HSV-1 vectors, many studies have documented the existence of known neural pathways linking transduced regions to the injection site. For example, transduction of the substantia nigra after striatal injection has been well documented for HSV-1 vectors (indicative of retrograde axonal transport from the injection site).[51,60,64,68,69,74,80] In addition, footpad injection of attenuated HSV-1 strains leads to transduction of the dorsal root ganglia,[81–85] which is highly indicative of axonal transport. Although the sensory ganglia and spinal cord are also common targets for HSV-1 gene transfer, they will not be considered further here, as this topic has been reviewed elsewhere.[28]

SOME HSV-1 STRAINS EXHIBIT A PREFERENCE FOR THE DIRECTIONALITY OF NEURONAL TRANSPORT

It has been demonstrated that different strains of HSV-1 show a predilection for neuronal transport in one direction over another. For example, injection of HSV-1 strain McIntyre-B into the motor cortex of the monkey resulted in retrograde transport, whereas injection of strain H129 gave anterograde transport.[86] Strain 17*syn*+ moves principally in the retrograde direction, although if introduced into gray matter, it can accomplish anterograde transport.[87] However, it is interesting to note that upon reaching the synapse after anterograde spread, this strain reverts to retrograde spread.[88] Why different strains prefer directional spread is not well understood, but may have to do with strain-specific differences in the viral proteins that mediate the entry and/or transport processes. Virus strains might preferentially bind to and enter neurons at the dendrites of the cell body *versus* the axon terminus, and thus may be more likely to undergo anterograde or retrograde transport, respectively. When planning to target a specific brain region for transduction, the HSV-1 strain to be used will be important to consider in making the recombinant vector.

In order to achieve gene expression after anterograde transport, the vector has to cross the synapse and enter another cell body. We have previously shown evidence for vector gene expression following movement in an anterograde direction with a vector strain (ICP34.5 mutant) that is unable to replicate in neurons.[89] Thus, attenuated HSV-1 vectors that are unable to replicate in neurons may be able to traverse at least a single neuronal synapse. Traversal of synapses has the potential to greatly increase the scope of transduction and hence enhance the correction of disease. This study provided no direct evidence for transduction of third-order neurons (movement across a second synapse). The prevalence of multiple-order neuronal transport by attenuated HSV-1 strains is currently unclear and warrants further investigation. For example, it is unknown if the first cell must support replication and/or die in order to achieve this type of spread. Furthermore, sensory neurons can release virus to infect surrounding neurons whereas the primary cell remains viable (as evidenced by the ability of that cell to support reactivation and transport of virus back to the periphery).[33]

CAN WE PREDICT WHERE AN HSV-1 VECTOR WILL TRAFFIC IN THE BRAIN?

Studies of HSV-1 vectors vary widely in the conditions used, making it difficult to directly compare the resulting transduction patterns produced. Some of the differences include: the type of vector (replication competent, replication defective, or amplicon), the parent strain of the vector (more/less virulent or preference for spread in a particular direction), the type of transgene encoded within the vector (secreted or non-secreted protein product), and the promoter used to drive transgene expression (viral or eukaryotic). Additionally, the animal model, the target tissue (central or PNS), the anatomical region targeted, the route (direct or peripheral), volume and titer injected, and the time point(s) analyzed also vary between studies. Lastly, not all brain regions are analyzed for transduction in each study; thus other regions may have been transduced that are not reported. The promoters used and time points analyzed are important to consider because many promoters are down-regulated during the onset of HSV-1 latency, thus transgene expression may be undetectable at later time points.[70,90,91]

Table 1 includes a summary of the HSV-1 gene transfer studies performed to date, with emphasis on the regions transduced and as a function of the injection site. Information on the type of HSV-1 vector, the animal model used, the time points analyzed, and the promoter used to drive transgene expression are included. The injection sites detailed in **Table 1** are diagrammed in a sagittal brain section in **Figure 3**. The caudate putamen (striatum) is the most common region targeted for HSV-1 vectors in the literature. The substantia nigra, thalamus, and various cortical regions are generally the areas to which the vector is transported after injection into the caudate putamen with a variety of different vectors, whether replication competent,[64,65,69,71,75,80,89] replication defective,[60,51] or amplicon vectors.[76,77]

HSV-1 VECTOR TROPISM CAN BE MODIFIED

Some reports have indicated that HSV-1 tropism can be changed by the deletion or modification of HSV-1 surface glycoproteins which normally direct binding and entry of the virus. Examples include deletions in gB and/or gC,[92] deletion of gD and replacement with vesicular stomatitis virus glycoprotein G,[93] and the creation of gC chimeras that contain ligands for the

Table 1 Summary of brain regions transduced by HSV-1 vectors

Injection site	Region of latency/transduction	HSV-1 vector type	Species	Promoter	Time point	Reference
Caudate putamen	Caudate putamen, cortex (bihemispheric), and substantia nigra	ICP0 mutant	Rat	ICP0	1–70 days	[80]
Caudate putamen	Caudate putamen	tk mutant	Rat	tk	3 days	[91]
Caudate putamen	Olfactory bulb, cortex, and septum	γ34.5 mutant	Mouse	LAT	>30 days	[65]
Caudate putamen	Caudate putamen, substantia nigra	ICP27, ICP4, γ34.5, and VP16 mutant	Rat	LAT	3 days–1 month	[51,58]
Caudate putamen	Substantia nigra	LAT, ICP4 mutant	Rat	MMLV LTR	4 days	[68]
Caudate putamen	Cortex, substantia nigra	γ34.5 mutant	Rat	LAT	1–30 days	[64]
Caudate putamen	Cortex, substantia nigra	γ34.5 mutant	Mouse/Rat	LAT	1–7 days	[69]
Caudate putamen	Cortex (bihemispheric), thalamus, and substantia nigra	gH, tk mutant	Rat	LAT	2–180 days	[60]
Caudate putamen	Cortex, thalamus, substantia nigra, and caudate putamen	VP16, ICP4, and ICP0 mutant	Rat	LAT	14–180 days	[60]
Caudate putamen	Caudate putamen, projecting frontal cortex	HF strain	Rat	LAT	45 days	[71]
Caudate putamen	Caudate putamen, substantia nigra, ventral tegmental area, and cortex	Amplicon	Rat	CMV IE	5 days	[74,76]
Caudate putamen	Caudate putamen, cortex	Amplicon	Rat	Various	4 days–2 months	[77]
Caudate putamen	Thalamus, hypothalamus	γ34.5 mutant	Mouse	LAT	4–43 days	[75]
Caudate putamen	Ipsilateral caudate putamen	Amplicon	Rat	Modified neuro-filament	5, 8, and 14 months	[99]
Caudate putamen	Cortex (bihemispheric), septum, thalamus, hypothalamus, midbrain, pons, medulla, and cerebellum	γ34.5 mutant	Mouse	LAT	2 months	[89]
Hippocampus	Cortex (bihemispheric), hindbrain, brainstem, and cerebellum	γ34.5 mutant	Mouse	LAT	>30 days	[65]
Hippocampus	Hippocampus, supramammillary bodies, septum, raphe nuclei, and entorhinal cortex	LAT, ICP4 mutant	Rat	MMLV LTR	4 days	[68]
Dorsal hippocampus	Septum, cortex, striatum, hippocampus, midbrain, and cerebellum	γ34.5 mutant	Mouse	LAT	2 months	[89]
Hippocampus	Hippocampus	ICP4 mutant	Rat	MMLV LTR	4 days–6 months	[101]
Dentate gyrus	Dentate gyrus, supramammillary nuclei, locus coeruleus, raphe nucleus, and periaqueductal raphe	Amplicon	Rat	CMV IE	5 days	[74,76]
Cerebellum	Mostly brainstem	γ34.5 mutant	Mouse	LAT	>30 days	[65]
Cerebellum	Cerebellum	Amplicon	Rat	HSV IE4/5	40 days	[120]
Cerebellar cortex	Cerebellar cortex, locus coeruleus, and pontine nuclei	Amplicon	Rat	CMV IE	5 days	[74,76]
Cerebral cortex	Olfactory bulb, septum, midbrain, hindbrain, brainstem, and cerebellum	γ34.5 mutant	Mouse	LAT	>30 days	[65]
Visual cortex	Cortex, caudate putamen, thalamus, midbrain, and hippocampus	γ34.5 mutant	Mouse	LAT	2 months	[89]
Somatosensory cortex	Caudate putamen, septum, thalamus, pons, medulla, and cortex	γ34.5 mutant	Mouse	LAT	2 months	[89]
Lateral ventricle	Cortex, septum, thalamus, hypothalamus, midbrain, hindbrain, pons, medulla, and caudate putamen	γ34.5 mutant	Mouse	LAT	2 months	[89]
Intrathecal	Midbrain, septum, midbrain, pons, medulla, cerebellum, and spinal cord	γ34.5 mutant	Mouse	LAT	2 months	[89]
Thalamus	Retinal ganglion neurons	ICP27, ICP4, γ34.5, and VP16 mutant	Rat	LAT	3 days–1 month	[51,58]
Medial septum	Septum	LAT, ICP4 mutant	Rat	MMLV LTR	4 days	[68]

Table 1 continued on the following page

Table 1 Continued

Injection site	Region of latency/transduction	HSV-1 vector type	Species	Promoter	Time point	Reference
Medial septum	Medial septum, cerebral cortex	ICP4 mutant	Rat	MMLV LTR	9 weeks	[78]
Olfactory bulb	Septum, midbrain, hindbrain, and brainstem	γ34.5 mutant	Mouse	LAT	>30 days	[65]
Substantia nigra	Globus pallidus	LAT, ICP4 mutant	Rat	MMLV LTR	4 days	[68]
Medial forebrain bundle	Medial forebrain bundle, substantia nigra, and parabrachial nuclei	gH, tk, VP16, ICP4, and ICP0 mutant	Rat	CMV IE or HSV LAT	2–5 days or 5–14 days	[70]
Lip	Trigeminal ganglia, spinal cord, and facial motor nucleus	SC16 (clinical isolate)	Mouse	LAT	6–720 days	[96]
Ear pinna	Facial and hypoglossal nuclei, and upper cervical spinal cord	SC16	Mouse	LAT	72–307 days	[67]
Ear pinna	Brainstem, spinal cord	SC16	Mouse	CMV IE or HSV LAT	5–365 days	[72]
Corneal scarification	Trigeminal ganglia, brainstem	Strain 17+	Mouse	LAT	4–126 days	[73,116]
Spinal cord	Spinal cord, brainstem	ICP27, ICP4, γ34.5, and VP16 mutant	Rat	LAT	3 days–1 month	[51,58]

CMV, cytomegalovirus; HSV, herpes simplex virus; LAT, latency-associated transcript; MMLV LTR, Moloney murine leukemia virus long terminal repeat; tk, thymidine kinase. A variety of different sites in the brain have been tested for vector inoculation, and patterns of latency and/or transgene expression have been documented. In general, whenever a careful analysis of transduction patterns has been performed, HSV-1 vectors have been shown to transduce a variety of brain regions. This is the case regardless of the HSV-1 strain, the transgene, the promoter used, or the inoculation site. The information is organized by injection site, and information on the parameters of each experiment is given to allow for comparison of the results.

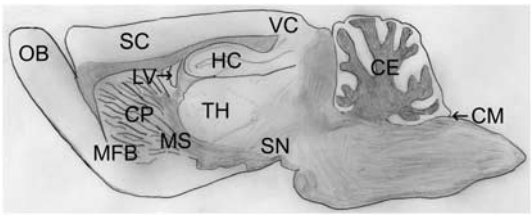

Figure 3 Regions of the rodent brain that have been injected with HSV-1 vectors. A sagittal section of the rat brain illustrates the regions previously tested for intracranial injection with HSV-1 gene transfer vectors. Key: olfactory bulbs (OB), medial forebrain bundle (MFB), somatosensory cortex (SC), caudate putamen/striatum (CP), lateral ventricle (LV), medial septum (MS), thalamus (TH), hippocampus (HC), substantia nigra (SN), visual cortex (VC), cerebellum (CE), cisterna magna (CM).

brain-specific factors glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor. These vectors achieved a modestly enhanced transduction of nigrostriatal neurons as compared to vectors using wild-type HSV-1 entry proteins.[94]

HSV-1 CAN TRANSDUCE MULTIPLE TYPES OF NEURONS

As HSV-1 is likely to be useful for long-term transduction to neurons, it is important to discuss which types of neurons can support HSV-1 latency. Although sensory neurons are the natural reservoir of latency, gene transfer experiments have shown that HSV-1 can also establish a latent infection (as defined by production of LATs) in other neurons within the brain,[59,60] as well as motor neurons.[95] However, there is at least some evidence to suggest that the accumulation of LATs is not equivalent in all subsets of neurons, and that it is the highest in sensory neurons.[96] As transgenes are commonly inserted into the LAT gene, it will be important to determine if long-term expression of transgenes is possible in these various types of neurons.

SOME BRAIN REGIONS MAY BE PREFERENTIALLY TARGETED FOLLOWING INTRACRANIAL INJECTION

Interestingly, work in our laboratory has shown that some regions of the brain are consistently targeted for HSV-1 gene transfer regardless of the intracranial injection site. Specifically, latency was consistently seen in the diencephalon, midbrain, and brainstem when either strain 1716 (an ICP34.5 mutant) or vectors based upon 1716 were injected into various regions of the brain ranging from the olfactory bulbs to intrathecal injection into the cisterna magna.[65,75,89] Consistent transduction of the midbrain has also been reported with peripheral inoculation of other ICP34.5 vectors.[79] The reasons for this finding are still unclear. However, some of these regions are known to become latently infected following normal HSV-1 infection in humans.[61] This may indicate that HSV-1 naturally targets particular brain regions for latency.

It is important to note that not all cells that harbor the HSV-1 genome produce detectable levels of the LATs.[97,98] Thus, detection of LATs or use of the LAT promoter to drive transgene expression may not faithfully reflect the number of cells that actually harbor the vector genome. In addition, studies using different vector constructs have shown that some latently infected cells produced the stable 2 kb intron (a marker for LAT gene expression and latency) but no detectable translation of complementary DNAs encoded within the LAT messenger RNA.[89,91] This phenomenon reduces the extent of transduction as measured by detection of the reporter enzyme. The mechanism is not well understood, but indicates a possible block to translation of LAT RNA in certain cell types.

SOME HSV-1 VECTORS DO NOT EFFICIENTLY TRANSDUCE THE AREA OF THE INJECTION SITE

Interestingly, it has been repeatedly noted that very little transduction occurs at the injection site with some HSV-1 vectors.[51,75,89] This result is the opposite of that seen with other viral vectors. The mechanism for this lack of transduction has yet to be fully elucidated, although it has been hypothesized that viral gene expression leads to toxicity and elimination of transduced cells at the injection site.[51] A side by side comparison of multiple HSV-1 vectors indicated that vectors with more immediate-early genes deleted (and hence decreased ability to execute the viral replication cycle) transduce the area of the injection site more efficiently than vectors that do express immediate-early genes.[51,60] An amplicon vector has been shown to efficiently transduce cells at the injection site,[99] whereas the non-pathogenic 1716 replicating virus generally does not transduce cells at the inoculation site, but is transported to neuronal cell bodies that project into the inoculation site. This suggests that vectors that do not express viral genes are able to transduce the injection site more effectively. With the non-pathogenic 1716 strain, there is little evidence of cytopathology at the inoculation site,[75] and this strain also replicated widely in tumor cells in the brain without damaging adjacent normal neurons.[100] Damage may be seen from replication of wild-type herpesviruses when used for track tracing[49] but that is because they use highly virulent viruses such as pseudorabies virus that would not be used for gene therapy applications.

Table 1 shows that injection into the caudate putamen often results in transduction of the same region, even with vectors that express viral genes. However, it should be noted that in most cases this transduction is only transient and very few positive cells could be found at later time points.[60,80] This is also true for the hippocampus.[101] Thus, it seems apparent that transduction of the injection site by vectors that express viral genes is only present at early time points and that this transduction does not persist into the latent stage. This may indicate a host immune response generated towards viral proteins or a response to cells damaged during the injection process. Whatever the mechanism, it is important to note that targeting a specific region for transduction may not be effectively accomplished with HSV-1 vectors by injecting directly into that site.

SCALING UP GENE TRANSFER FROM A SMALL TO A LARGE BRAIN

A major issue in gene therapy for the brain is scaling up successful methods in the rodent to larger brains. Studies performed in the brain of larger animals with the non-neurotropic vectors (*e.g.*, adenovirus, AAV, or lentivirus) have shown that the transduction patterns seen in rodents do not directly scale-up in the larger brain, for example the total distance of transduction from the injection site may be similar in either a small or large brain (*e.g.*, cat or monkey).[20,102,103] This indicates that in order to target widespread regions of a large brain with these vectors, many injections would have to be performed. Although one study in the cat estimated that 350 well-spaced injections might be necessary to transduce an entire child's brain with an AAV vector,[20] a subsequent study found that only about 1/10 this number might be sufficient for a secreted protein.[104]

Although most of the HSV-1 neural tracing studies have been performed in rodents, some were in larger animals, such as monkeys.[49] A comparison of studies performed in rodents and monkeys indicates that HSV-1 may spread similarly in both small and large brains. For example, injection into the visual cortex of either the rat or cebus monkey led to infection of specific regions of the thalamus and superior colliculus that are known to send projections to the visual cortex.[105,106] Thus, the patterns of spread of HSV-1 may indeed be similar in small and large brains, even though the relative distances are very different. However, there is relatively little information available in the literature for HSV-1 spread in large brains. Thus, it is unclear if this pattern of scaling up from small to large brains will be a common occurrence.

Another important point to consider when comparing these studies is that the ability to detect viral antigens during the acute stage of infection (tract-tracing) is not necessarily indicative of where the virus will establish latency (a common endpoint for gene transfer). Expression of a therapeutic gene during the acute stage of infection alone is unlikely to have any long-term impact on a lifelong disorder such as a genetic disease. Thus, analyses must be performed during the latent period. However, our analysis of the latent regions established with attenuated vectors[89] seems to correlate well with tracing studies of others performed at 3–4 days post-infection using wild-type virus.[49] Only limited work has been carried out to map regions of HSV-1 latency in the large animal brain. One study was performed where a neuro-attenuated HSV-1 strain was injected unilaterally into the dog brain, following which latently infected cells were detected in the contralateral hemisphere by LAT *in situ* hybridization.[107] Detection of positive cells in the contralateral hemisphere represents a substantial distance from the injection site in the dog brain (estimated to be 1–3 cm), and indicates that even attenuated HSV-1 has the ability to both spread and establish latency over large distances when introduced into a larger brain. However, the analysis was limited to regions proximal to the injection site in that study and no information was given on movement in the rostral/caudal axis. Clearly, further investigation is needed in order to determine the extent of transport of attenuated HSV strains in a larger brain.

LYSOSOMAL STORAGE DISORDERS ARE GOOD MODELS FOR GENE THERAPY OF THE BRAIN

The LSDs are caused by genetic defects in lysosomal enzymes that result in accumulation of substrates in the lysosomes. Over 50 LSDs exist and the group of diseases has a collective occurrence of about one in 7,000.[108] Most LSDs have a neurological component, although the extent and distribution of the storage pathology in the brain differs amongst the various disorders.[109] There are many animal models of the human LSDs, both in rodents and larger domestic animal species.[22] The brain is generally refractory to peripheral enzyme delivery strategies such as enzyme replacement or bone marrow transplantation, unless performed early in development.

The basis for treating most LSDs is that lysosomal enzymes are secreted from producing cells and can be taken up by adjacent cells, via mannose-6-phosphate receptor-mediated endocytosis. This results in catabolism of the stored substrate.[110–112] Thus, the gene needs to be transferred to only a fraction of cells in any target organ in order to achieve correction of many cells.[12] Additionally, neuronal transport of some lysosomal enzymes has been documented, which can facilitate distribution of the normal enzyme to more distal sites.[10,113–115] Finally, only a small percentage of normal lysosomal enzyme activity is often sufficient to correct lysosomal storage.[116,117] As vector targeting is currently insufficient to transduce all CNS cells, the secretory nature of many of the lysosomal enzymes indicates that these diseases are particularly amenable to treatment even with the limitations associated with the current gene transfer vectors.

Early experiments showed that an HSV vector could deliver a lysosomal enzyme from the PNS to the CNS and correct the enzyme deficiency in a few cells[73] by expressing it from the LAT promoter. Engineering the promoter/transgene design and direct injection of a vector with a non-pathogenic backbone (HSV 1716) led to greatly increased and longer term expression[59,75] which mediated widespread correction of storage.[89]

FOCAL DISEASES CAN ALSO BE TREATED BY HSV-1 GENE THERAPY

Some brain diseases that are amenable to gene therapy are localized to particular regions of the brain; examples of these types of disorders include Parkinson's disease and brain tumors. Several studies have shown success in treating a rat of model of Parkinson's disease.[99,118] Interestingly, these studies have used non-replicating vectors which may be more useful for local therapies because they effectively transduce the area inoculated. In addition, human clinical trials against glioblastoma using conditionally replicating HSV-1 strains (but not encoding a transgene) indicated that these vectors are safe for use in humans because they did not cause encephalitis.[63,119]

SUMMARY

The ability of attenuated HSV-1 vectors to spread to regions distant from the injection site indicates that the use of a limited number of injection sites will successfully transduce a major portion, if not an entire large brain. However, many questions remain to be answered. Insufficient data is currently available to allow prediction of where a vector will go following injection into a particular site. Further studies to determine if the vector transport documented in small animal brains will scale-up to large animal brains are needed. Are there any brain regions that are refractory to transduction by HSV-1? Can the virus be effectively targeted to certain brain regions while avoiding others? Can the virus remain transcriptionally active in all regions of the brain? Clearly, the potential for widespread brain transduction is an exciting possibility, but much more work will be required to realize this potential.

ACKNOWLEDGMENTS

This work was supported by NIH R01 NS29390 and T32 AI07324.

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