HSV Recombinant Vectors
General Characteristics and Potential for Use in the Central Nervous System

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1. INTRODUCTION

Herpes simplex virus type 1 (HSV-1) offers a number of distinct advantages over other viruses for development as a gene transfer tool. These include its wide host range and high efficiency of gene transfer, as well as its potential for incorporating a large payload (15–30 kb) of foreign DNA. The HSV-1 genome itself is large (150 kb encoding 80-plus genes) and can be cumbersome to manipulate, but there exists a rich source of mutants and genetic information as a result of decades of intensive study by investigators worldwide (for review, see ref. 1). The viral genome consists of double-stranded linear DNA and is divided into long and short unique regions each flanked by their own repeated sequences. In nature, HSV undergoes either a lytic or a latent infection. During the lytic infection, HSV genes are expressed in a temporally regulated cascade consisting of three main phases: immediate-early (IE), early, and late. The latent infection occurs in sensory ganglia and is characterized by a complete absence of lytic gene expression but for the synthesis of a set of transcripts (of unclear function) referred to as the latency-associated transcripts (LATs). The potential applications for an HSV vector could be for either short- or long-term gene delivery and expression, and need not be limited to neuronal targets. For example, HSV mutants with selective growth phenotypes are currently showing much promise in the treatment of cancer, as discussed elsewhere in this volume. The focus of this chapter is to consider the suitability of recombinant HSV vectors for long-term gene transfer, and to discuss the areas of development that will be required to achieve this aim (for a more comprehensive review treating HSV-1 vector development, see ref. 2).

For the purposes of long-term gene transfer, HSV has a number of desirable properties, including the ability to remain latent during a natural infection for the lifetime of an individual, express transcripts from a portion of its genome, and avoid inducing an immune response. However, despite much tinkering with the viral genome, cytotoxicity and shut-down of transgene expression remain very significant problems. Recent efforts have demonstrated that a reduction in cytotoxicity is achievable, but at the cost of reduced transgene expression. Thus, any hopes that recombinant HSV vectors might be useful for gene replacement therapy in the brain must be offset by the reality that it is
currently uncertain how to achieve a useful level of long-term gene expression. HSV amplicon vectors may offer a potential alternative. These are essentially plasmids that can be replicated and packaged by a helper virus (as discussed in Chapter 4). The recent development of a helper-free amplicon system has demonstrated the advantages of a herpes vector without viral genes (3). However, there are currently formidable problems associated with the efficient production of HSV amplicon vectors that need to be solved. In the meantime, further study of the mechanisms that cause the shutoff of viral gene expression will be critical to aid rational design of recombinant viral vectors suitable for long-term gene transfer.

2. TOXICITY

2.1. Replication-Attenuated Mutants

HSV vectors have been derived from mutants that can be broadly split into two categories: replication-competent and replication-defective. Some investigators have utilized replication-competent mutants that have a limited ability to replicate in nondividing cells or that cannot replicate at a low multiplicity of infection (MOI) as a means to achieve more widespread dissemination in vivo than nonreplicating vectors. These have included viruses with attenuating mutations in genes encoding ICP0, thymidine kinase, and the US3 protein kinase (4–6). Although inoculation of animals with such vectors rarely causes gross defects, on a more local level viral replication itself invariably does induce a degree of cell damage (6). For this reason, attention has been more focused on mutants that are completely defective for replication.

2.2. ICP4 Mutants

Mutants that have a large deletion in an essential gene have a smaller probability of reverting to a replication-competent phenotype than temperature-sensitive mutants, and can be grown on specially constructed cell lines that provide the viral gene product in trans. Deletion mutants in ICP4, the major transcriptional activator protein of HSV-1 required for expression of early and late viral genes, have been considered attractive candidates as vectors, since they express few other viral genes (7,8). Growth of an ICP4 mutant, such as D30EBA (8), on the ICP4-complementing E5 cell line (9) generates replication-competent virus as a result of rescue of the cellular copy of the ICP4 gene at a frequency of less than 1 in $10^6$. Vectors derived from ICP4 mutants have been used to achieve high-level, but transient transgene expression in the central nervous system (CNS) in vivo (4), and in culture neurons in vitro (10). Longer-term expression has been observed in the peripheral nervous system (PNS) (11). However, despite the fact that an ICP4 deletion mutant does not replicate and expresses few other viral genes, cell survival in vitro is markedly impaired as a result of cytopathic effects (CPE), which occur by 1–3 d postinfection (10,12). It is harder to determine the fate of individual infected cells in vivo, especially if transgene expression is not detectable. Although an ICP4 mutant is able to establish latency in sensory neurons (13), the dramatic loss of viral DNA that occurs in the brain soon after infection is consistent with the occurrence of in vivo toxicity (14). Potential causes for the cytotoxicity of HSV vectors include nonviral factors present in the vector stock, components of the virion, and genes expressed by the vector.