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Properties of a herpes simplex virus multiple immediate-early gene-deleted recombinant as a vaccine vector

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Abstract

Herpes simplex virus (HSV) recombinants induce durable immune responses in rhesus macaques and mice and have induced partial protection in rhesus macaques against mucosal challenge with virulent simian immunodeficiency virus (SIV). In this study, we evaluated the properties of a new generation HSV vaccine vector, an HSV-1 multiple immediate-early (IE) gene deletion mutant virus, *d*106, which contains deletions in the *ICP4*, *ICP27*, *ICP22*, and *ICP47* genes. Because several of the HSV IE genes have been implicated in immune evasion, inactivation of the genes encoding these proteins was expected to result in enhanced immunogenicity. The *d*106 virus expresses few HSV gene products and shows minimal cytopathic effect in cultured cells. When *d*106 was inoculated into mice, viral DNA accumulated at high levels in draining lymph nodes, consistent with an ability to transduce dendritic cells and activate their maturation and movement to lymph nodes. A *d*106 recombinant expressing *Escherichia coli* β-galactosidase induced durable β-gal-specific IgG and $CD8^+$ T cell responses in naive and HSV-immune mice. Finally, *d*106-based recombinants have been constructed that express simian immunodeficiency virus (SIV) gag, env, or a rev-tat-nef fusion protein for several days in cultured cells. Thus, *d*106 shows many of the properties desirable in a vaccine vector: limited expression of HSV gene products and cytopathogenicity, high level expression of transgenes, ability to induce durable immune responses, and an ability to transduce dendritic cells and induce their maturation and migration to lymph nodes.

Keywords: Replication-defective virus; Vaccine vector; SIV

Introduction

Viral recombinant strains expressing heterologous proteins have been tested widely as vaccine vectors against a number of virus infections (Murphy and Chanock, 2001), and a number of viruses, including pox viruses, adenoviruses, parvoviruses, alpha viruses, and herpes viruses have been utilized. Despite the extensive testing of various vaccine strategies as AIDS vaccines, none have been very effective against the pathogenic simian immunodeficiency virus (SIV) strains in nonhuman primates in the infection models that most closely approximate human immunodeficiency virus (HIV) infection of humans. Thus, there is a continuing dire need to design and test novel vaccine approaches as candidate AIDS vaccines.

We have previously used herpes simplex virus 1 recombinants as vaccine vectors expressing SIV envelope protein and have shown that these recombinants could induce partial protection against mucosal SIVmac239 infection in rhesus macaques (Murphy et al., 2000). One of these vectors, HSV-1 *d*27, was a replication-defective mutant strain that was defective for the *UL54* (*ICP27*) gene. In this study, we have attempted to

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improve upon that first-generation vaccine vector by the use of recombinant HSV-1 strains that contain additional mutations beyond the *ICP27* gene mutation.

Herpes simplex virus productive infection involves the expression of a series of kinetically regulated groups of viral genes (Roizman and Knipe, 2001). Viral immediate-early (IE) genes are transcribed immediately upon viral entry without prior viral protein synthesis. Three of the IE proteins, ICP4, ICP0, and ICP27, stimulate later viral gene expression. The next set of genes expressed, the early genes, encode viral proteins mostly involved in viral DNA replication. Following viral DNA replication, the late viral genes are expressed optimally. HSV infection shuts down host transcription, RNA splicing, and protein synthesis. Inhibition of host cell protein synthesis requires both the virion host shutoff (vhs) function and the IE ICP27 protein (Smiley, 2004; Song et al., 2001). Inhibition of host transcription requires both ICP4 and ICP27 (Spencer et al., 1997) whereas inhibition of RNA splicing requires ICP27 (Hardy and Sandri-Goldin, 1994).

HSV recombinants have a number of properties that would be advantageous for a vaccine vector. HSV can infect a wide range of tissues and host species and can generate immune responses by various routes of inoculation, including mucosal (intranasal or intravaginal) administration. HSV induces durable immune responses, in part due to latent infection and reactivation, but replication-defective mutant HSV strains induce equally durable immune responses (Morrison and Knipe, 1994). Herpesviral recombinants can accommodate sizeable inserts of heterologous DNA for vectoring purposes (Knipe et al., 1978) and induce Th1-biased cellular responses (Brubaker et al., 1996; Nguyen et al., 1994), which are desirable properties for a vaccine vector. HSV-1 activates TLR2 (Kurt-Jones et al., 2004, 2005) to induce a pro-inflammatory cytokines as well as activating TLR9 to induce type I interferons (Krug et al., 2004; Lund et al., 2003). Also, an HSV replication-defective vector expressing β-galactosidase showed full immunogenicity in the presence of pre-existing anti-HSV immunity (Brockman and Knipe, 2002).

However, HSV recombinants express a large number of HSV proteins that could either evade or blunt the host immune response or compete for immunogenicity with a vector-expressed heterologous antigen. Therefore, mutating the *ICP4* or *ICP27* genes could improve the immunogenicity of the transgene product by reducing the number of HSV gene products expressed. Similarly, ICP4 and ICP27 inhibit the transcription of host genes, and this could decrease antigenic presentation by down-regulating MHC class I in infected cells, for example. HSV ICP47 has been shown to block TAP transport of peptides into the ER for loading onto MHC class I (York et al., 1994) whereas ICP22 has been reported to inhibit MHC class II presentation (Barcy and Corey, 2001). Thus, mutational inactivation of these genes would be expected to increase the immunogenicity of a vaccine vector. An additional approach that addresses these issues is the use of HSV amplicon or replicon strains (Spaete and Frenkel, 1982) as vaccine vectors. HSV-1 amplicons expressing HIV envelope are immunogenic in mice (Gorantla et al., 2005; Hocknell et al., 2002) but no information is available about immunogenicity in primates. Production issues may limit this approach using current technology.

Samaniego et al. (1998) studied the properties of a number of recombinant HSV-1 strains that are defective for various permutations of IE genes. A recombinant virus in which all IE genes were inactivated showed the lowest cytopathic effect on host cells but very low expression of the GFP transgene. The *d*106 recombinant strain, which expresses only one IE protein, ICP0, showed the highest GFP transgene expression balanced with less cytopathogenicity than many of the other strains. The *d*106 virus contains deletions of the *ICP4* and *ICP27* ORFs and contains deletions of the promoter/enhancers of the *ICP22* and *ICP47* genes (Samaniego et al., 1998). The *d*106 virus can be grown in cell lines that contain the *ICP4* and *ICP27* genes, which are activated upon viral infection and complement the defects in those genes. Expression of ICP4 enhances expression of ICP22, allowing enhanced growth of the virus in the cells. Because of the potential for expression of the transgene and limited cytopathogenicity, we have chosen the *d*106 strain for study as a second-generation HSV-1 replication-defective vaccine vector.

Results

Properties of d106 virus as a vaccine vector

We have previously used an HSV-1 replication-defective *ICP27* (*UL54*) gene mutant, HSV-1 *d*27, as a vaccine vector for expression of SIV proteins and immunization of rhesus macaques, resulting in protection of some of the monkeys against mucosal challenge infection with pathogenic SIV mac239 virus (Murphy et al., 2000). To try to improve upon the *d*27 vector, we tested a virus with additional IE gene mutations, the *d*106 mutant strain (Samaniego et al., 1998). HSV-1 *d*106 (Fig. 1A) contains deletions inactivating four of the five IE genes (*ICP4*, *ICP27*, *ICP22*, and *ICP47*) and has an expression cassette containing the green fluorescent protein (GFP) gene under the control of the human cytomegalovirus (HCMV) IE promoter-enhancer inserted into the *ICP27* gene locus as a model transgene (Fig. 1A). ICP4 is the major transactivator of E and L viral genes; therefore, deletion of the *ICP4* gene would decrease HSV gene expression, reducing antigenic competition with the transgene products. ICP47 binds to the TAP peptide transporter and blocks peptide transport into the endoplasmic reticulum and loading of MHC class I, thereby blocking MHC class I presentation (York et al., 1994). ICP22 has also been reported to inhibit MHC class II presentation (Barcy and Corey, 2001); therefore, its lack of expression should also improve immunogenicity of the vector.

Samaniego et al. (2001) compared a number of HSV-1 strains that were mutated for varying numbers of HSV IE genes. They found that *d*106 showed more GFP transgene expression than other mutant strains. We therefore chose this strain for testing as a vaccine vector. Because Samaniego et al. (2001) had observed that *d*106 infection caused a reduction in colony formation in infected Vero cells, we first examined the cytopathic effect and host protein synthesis shutoff induced