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Effect of promoter strength on protein expression and immunogenicity of an HSV-1 amplicon vector encoding HIV-1 Gag

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Abstract

Helper-free herpes simplex virus type-1 (HSV-1) amplicon vectors elicit robust immune responses to encoded proteins, including human immunodeficiency virus type-1 (HIV-1) antigens. To improve this vaccine delivery system, seven amplicon vectors were constructed, each encoding HIV-1 Gag under the control of a different promoter. Gag expression levels were analyzed in murine and human cell lines, as well as in biopsied tissue samples from injected mice; these data were then compared with Gag-specific T cell responses in BALB/c mice. The magnitude of the amplicon-induced immune response was found to correlate strongly with the level of Gag production both *in vitro* and *in vivo*. Interestingly, the best correlation of the strength of the amplicon-induced immune response was with antigen expression in cultured DC rather than expression at the tissue site of injection or in cultured cell lines. These findings may have implications for the generation of improved HSV-1 amplicon vectors for HIV-1 vaccine delivery.

Keywords: HSV-1 amplicon vectors; Transcriptional control elements; HIV vaccines; Gag; Dendritic cells (DC)

1. Introduction

Helper-free herpes simplex virus type-1 (HSV-1) amplicons represent a replication-defective gene transfer vector system with several biological features that make them an attractive choice for vaccine delivery applications [1,2]. Among the appealing characteristics of these vectors are their large insert capacity, favorable safety profile and broad cellular/tissue tropism which extends to antigen presenting cells, such as dendritic cells (DC) [3]. As a result, helper-free HSV-1 amplicons encoding mammalian or microbial proteins can elicit strong, antigen-specific immune responses, and may have utility in a range of applications, including cancer treatment and prophylactic vaccination against pathogens such as human immunodeficiency virus type-1 (HIV-1) [3–10].

Considerable attention has been paid to the rational improvement of amplicon vectors for gene transfer applications in the central nervous system (CNS) [11–15], but systematic attempts to enhance the performance of amplicon-based vaccine vectors have been more limited. We therefore sought to carefully characterize the effect of various transcriptional control elements on amplicon-driven expression

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of an HIV-1 antigen (Gag), and also on the strength of the host immune response to that antigen, following vector delivery to BALB/c mice. As part of this analysis, we also examined whether the magnitude of the *in vivo* immune response was correlated with the level of antigen expression, either at the local tissue site of vector injection *in vivo*, or in cultured cells that were representative of key potential *in vivo* target cells (*i.e.*, fibroblasts, dendritic cells, keratinocytes and epithelial cells).

To date, most studies involving HSV-1 amplicon-vectored vaccines have relied on the use of the HSV-1 immediate-early 4/5 promoter (HSV IE4/5) [3–5,7]. However, it is unclear whether this is the ideal transcriptional control element for use in vaccine studies-especially since the activity of this promoter is strongly influenced by the HSV-1 tegument protein, VP16 [16–18]. We therefore generated a series of seven amplicon constructs, each of which contained the HIV-1 *gag* gene, under the transcriptional control of a different promoter element. The constructs included four promoters that were derived from the human cytomegalovirus (HCMV) immediate-early promoter (one wild-type promoter and three hybrid promoters, incorporating additional regulatory elements), as well as two retroviral promoters and the HSV IE4/5 promoter.

Amplicon vectors harboring the various promoters were used to transduce a panel of murine and human cell lines, and protein expression levels were assessed by immunoblot analysis and p24 Gag ELISA. The vectors were also injected into BALB/c mice, via an intradermal route, and Gag expression levels were measured at the local site of injection by p24 Gag ELISA. Finally, Gag-specific T cell immune responses were also analyzed. These studies revealed that constructs that contained two of the hybrid CMV promoters elicited the strongest immune responses, and significantly outperformed all of the other constructs. Interestingly, the magnitude of the *in vivo* immune response elicited by the various constructs was found to correlate significantly with the level of Gag expression both *in vitro* (in cultured cell lines and primary murine dendritic cells) and *in vivo* (in biopsied tissue from the local site of amplicon injection). These findings have implications for the rational improvement of HSV-1 amplicon-vectored vaccines.

2. Materials and methods

2.1. Transcriptional promoter elements

Seven helper virus-free HSV-1 amplicon vectors encoding HIV-1 Gag were developed, each with a different promoter (Fig. 1). These promoters included: two retroviral promoters (the Rous sarcoma virus [RSV] and the myeloproliferative sarcoma virus [MPSV] long terminal repeat [LTR]) as well as the major human cytomegalovirus immediate-early promoter [CMV], and several hybrid promoters derived from this element; these included a composite CMV/chicken β-actin promoter [CAG] [19], a modified CMV promoter containing regulatory sequences from the human T-cell leukemia virus type-1 LTR [CMV-R] [20], and a CMV promoter linked to the CMV Intron A sequence [CMV-I] [21,22].

2.2. Construction of HSV-1 amplicon vectors with different promoter elements driving HIV-1 gag expression

The HSV-1 amplicon contains the HSV-1 packaging signal and origin of DNA replication [2], inserted into the backbone of pVax (Invitrogen, Carlsbad, CA). The indicated gene cassettes (Fig. 1) were inserted into the plasmid polylinker. The basic CMV promoter/enhancer was used from a modified pVax-based amplicon plasmid and the gag insert cloned in with *Hin*dIII and *Xba*I. The CMV promoter/enhancer and the associated Intron A sequence were PCR amplified from plasmid VRC5309 (provided by the NIH VRC) using forward (5′-ACCATATGCACGCGTGTGTGAAATACCGCA-3′) and reverse oligonucleotide primers (5′-TGGCGATATCTC-TAGAGCGGCCGCGATATC-3′) [20,23] (VRC5309 was generously provided by Dr. Gary Nabel of the NIH Vaccine Research Center, VRC). The 1.6 kb PCR product was digested with *Mlu*I and *Xba*I and cloned into the amplicon backbone to create CMV-I. The CMV promoter/enhancer and the HTLV-1 R region together with the gag insert were excised from the plasmid VRC4401 (also provided by the NIH VRC [24]) using *Hin*dIII and *Xba*I. The resulting DNA fragment was cloned into the promoterless amplicon vector backbone to create CMV-R.

The CAG promoter was contained in a plasmid kindly provided by Dr. Joshi Jacob [19]. To construct the CAG-containing amplicon, the HIV-1 gag gene was first PCR amplified using forward (5′-ACTGGCGGCCGCCTCGAG-CACCATGGGCGCCCGCGCCAGCGTG-3′) and reverse oligonucleotide primers (5′-GACTTCTAGAAGATCTTTA-TTGTGACGAGGGGTCGCTG-3′). This PCR amplimer was then digested with *Not*I and *Xba*I, and inserted into the amplicon backbone cut with the same enzymes. The CAG promoter element was inserted into the amplicon backbone upstream of the *gag* gene, using *Nde*I and *Not*I.

The RSV promoter was contained in a commercially obtained plasmid (pREP4; Invitrogen, Carlsbad, CA), which was digested with *Bam*HI and *Hin*dIII to incorporate the *gag* insert that was digested using the same enzymes. The RSV-gag insert was PCR amplified using 5′-GGCGTTTAA-ACACTCTCAGTACAATCTGCTCTGATGCCGC-3′ forward primer and 5′-GCCTCGAGGAGCTCGGTACCTATC-TTT-3′ reverse primer; the resulting PCR fragment was cut with *Pme*I and *Xho*I and then ligated into the amplicon backbone that was digested with *Hinc*II and *Xho*I; this was then cloned into the amplicon backbone to create RSV. The MPSV [25,26] promoter was PCR amplified using 5′-GTTGACCCCGGGGCAAGGCATGGAAAATA-3′ forward primer and 5′-GCAAGCTTGGATCCATTG-AGAACACGGG-3′ reverse primer. The PCR product was