High-Level Expression of Glycoprotein D by a Dominant-Negative HSV-1 Virus Augments its Efficacy as a Vaccine against HSV-1 Infection

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Using the T-REx (Invitrogen, Carlsbad, CA) gene switch technology, we previously generated a dominant-negative herpes simplex virus (HSV)-1 recombinant, CJ83193, capable of inhibiting its own replication as well as that of wild-type HSV-1 and HSV-2. It has been further demonstrated that CJ83193 is an effective vaccine against HSV-1 infection in a mouse ocular model. To ensure its safety and augment its efficacy, we generated an improved CJ83193-like HSV-1 recombinant, CJ9-gD, which contains a deletion in an HSV-1 essential gene and encodes an extra copy of gene-encoding glycoprotein D (gD) driven by the tetO-bearing human cytomegalovirus major immediate-early promoter. Unlike CJ83193, which exhibits limited plaque-forming capability in Vero cells and expresses little gD in infected cells, CJ9-gD is completely replication defective, yields high-level expression of gD following infection, and cannot establish detectable infection in mouse trigeminal ganglia following intranasal and ocular inoculation. Mice immunized with CJ9-gD produced 3.5-fold higher HSV-1 neutralizing antibody titer than CJ83193-immunized mice, and were completely protected from herpetic ocular disease following corneal challenge with wild-type HSV-1. Moreover, immunization of mice with CJ9-gD elicited a strong HSV-1-specific T-cell response and led to an 80% reduction in latent infection by challenge wild-type HSV-1 compared with the mock-immunized control.

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INTRODUCTION

The major clinical significance of herpes simplex virus type 1 and type 2 (HSV-1 and -2) is their ability to cause acute primary infection and to reactivate periodically from latency and cause recurrent infection. Although HSV infections are often asymptomatic, their clinical manifestations include orofacial infections, genital herpes, neonatal herpes, keratoconjunctivitis, and herpes encephalitis (Whitley *et al.*, 1998; Stanberry *et al.*, 2000; Koelle and Ghiasi, 2005). HSV-2 is the primary cause of genital ulcer disease. HSV-1 infection often associates with orofacial blisters and herpetic ocular disease, which is the leading cause of virus-induced blindness in developed as well as less developed countries (Group THEDS, 1998; Kovac-Kovacic and Skaleric, 2000; Xu *et al.*, 2002). Notably, there has been a significant increase in HSV-1-related genital herpes in recent years and in some developed countries or populations, HSV-1 infection has become a common cause of genital herpes (Lafferty *et al.*, 2000; Lowhagen *et al.*, 2000; Nilsen and Myrmel, 2000; Ribes *et al.*, 2001; Scoular *et al.*, 2002; Tran *et al.*, 2004; Roberts, 2005). Although the severity of most symptomatic HSV infections can be reduced by antiviral treatment, there is no effective medication that can prevent primary HSV infections nor decrease the incidence of recurrences except for daily suppressive therapy. Thus, there is a strong need for a safe and effective vaccine against HSV infections (Stanberry, 2004; Koelle and Corey, 2008).

HSV gene expression was classified into three major phases during productive infection, named immediate-early (α), early (β), and late (γ), with late genes being further divided into two groups, $\gamma 1$ and $\gamma 2$ (Roizman and Knipe, 2001). The expression of α genes requires no *de novo* protein synthesis and is activated by the virion-associated protein VP16 together with cellular transcription factors (Roizman and Knipe, 2001). Although the expression of viral α and β genes does not depend on viral DNA replication, expression of γ genes is highly influenced by *de novo* viral DNA synthesis. Specifically, *de novo* viral DNA replication leads to increased expression of $\gamma 1$ genes and inhibition of viral DNA replication blocks expression of $\gamma 2$ genes.

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Abbreviations: BM-DCs, bone marrow-derived dendritic cells; FBS, fetal bovine serum; gD, glycoprotein D; hCMV, human cytomegalovirus; HSV, herpes simplex virus; MOI, multiplicity of infection; SFCs, spot-forming cells; TG, trigeminal ganglia

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Safety and efficiency in eliciting an effective host immune response are two major criteria in developing recombinant viral vaccines against wild-type viral infections. In the last decade, various forms of HSV replication-defective viruses and neuroattenuated, replication-competent mutants have been tested as potential live vaccines against HSV infection in several different animal models (Meignier *et al.*, 1988; Meignier *et al.*, 1990; Nguyen *et al.*, 1992; Farrell *et al.*, 1994; McLean *et al.*, 1994; Morrison and Knipe, 1994; Boursnell *et al.*, 1997; Brehm *et al.*, 1997; Spector *et al.*, 1998; Walker and Leib, 1998; Da Costa *et al.*, 1999; Keadle *et al.*, 2002; Osorio and Ghiasi, 2003, 2005; Prichard *et al.*, 2005; Parker *et al.*, 2006). Given that both replication-defective viruses and neuroattenuated mutants are replication competent in the context of wild-type virus, their use as a vaccine in humans does pose a safety concern, particularly in individuals who harbor latent HSV infection (Koelle and Ghiasi, 2005). Using the T-REx (Invitrogen, Carlsbad, CA) gene switch technology developed in this laboratory and the dominant-negative mutant polypeptide UL9-C535C of HSV-1 origin binding protein UL9, we have established a new strategy for development of a safe and effective recombinant HSV vaccine against HSV-1 infection. Specifically, we constructed a replication-defective HSV recombinant, CJ83193, capable of inhibiting the replication of wild-type HSV-1 and HSV-2 in cell cultures (Yao and Eriksson, 1999, 2002) and in the central nervous system of mice coinoculated with HSV-1 and CJ83193 (H Augustinova and F Yao, unpublished data). We demonstrate further that CJ83193 is an effective vaccine against HSV-1 infection in mice and is capable of eliciting long-term humoral and cell-mediated immunity comparable with that induced by wild-type HSV-1 (Augustinova *et al.*, 2004).

HSV-1 encodes at least 12 glycoproteins, among which gB, gC, gD, gH, and gL are most abundantly expressed in infected cells and constitute the major HSV-1 glycoproteins on the viral envelope (Handler *et al.*, 1996). gD is the major target for neutralizing antibodies against HSV infection (Sim and Watson, 1973; Cohen *et al.*, 1984; Para *et al.*, 1985; Minson *et al.*, 1986). The role of gD in eliciting host T-cell response has also been illustrated (Zarling *et al.*, 1986a, b; Koelle *et al.*, 1994, 1998b; Mikloska and Cunningham, 1998). We have shown that the expression of gD, a γ gene product, is significantly lower in CJ83193-infected cells than cells infected with wild-type HSV-1. We thus hypothesize that the vaccine efficacy of CJ83193 can be elevated if the expression of gD can be substantially increased.

Here we describe construction of a CJ83193-derived dominant-negative and replication-defective HSV-1 recombinant, CJ9-gD, which has a deletion in the essential *UL9* gene and encodes an extra copy of the *gD* gene controlled by the tetO-bearing human cytomegalovirus (hCMV) major immediate-early promoter. Thus, unlike CJ83193, which exhibits very limited plaque-forming ability in Vero cells and expresses little gD, CJ9-gD is completely replication defective and capable of expressing high levels of gD in infected cells. We show that CJ9-gD is a safer and more effective vaccine than CJ83193 in a mouse model of HSV-1 infection.

RESULTS

Construction and *in vitro* characterization of CJ9-gD

To introduce the HSV-1 *gD* gene under control of the tetO-hCMV IE promoter into the CJ9-lacZ genome at the UL9 locus (Figure 1a), we transfected U2CEP4R-11 cells with 6 μg of Mfe I-linearized p9DNATO-gD and 0.1 μg of pcDNA-UL9 followed by CJ9-lacZ superinfection at a multiplicity of infection (MOI) of 5 PFU per cell. Progeny viruses were harvested at 24 hours postinfection and plaque assays were performed on RUL9-8 cell monolayers in the presence of neutral red and X-Gal. White plaques, indicating replacement of the *lacZ* gene by the *gD* gene-containing fragment of p9DNATO-gD, were isolated and plaque purified three more times on RUL9-8 cell monolayers. CJ9-gD is a viral recombinant obtained after four rounds of plaque purification that exhibits no blue plaques in plaque assay in the presence of X-Gal, and expresses no detectable gD in infected U2CEP4R-11 cells in the absence of tetracycline and a high level of gD in the presence of tetracycline (F Yao *et al.*, unpublished data), which demonstrates that the detected gD expression in CJ9-gD-infected U2CEP4R-11 cells is driven by the tetO-bearing hCMV major immediate-early promoter (Yao *et al.*, 1998). Western blot analysis (Figure 2a) shows that CJ9-gD expresses significantly higher levels of gD than CJ83193 and CJ9-lacZ in infected Vero cells. The replacement of the *lacZ* gene by the *gD* gene at the UL9 locus in CJ9-gD was confirmed by PCR analysis of CJ83193, CJ9-lacZ, and CJ9-gD viral DNA with the UL9 sequence-specific primers that flank the *lacZ* gene- and the *gD* gene-containing DNA insert (data not shown). No viral plaques were detected on Vero cell monolayers when a total of 1.1×10^8 PFU of CJ9-gD virus was assayed, whereas an average of 1 plaque per 1×10^6 PFU of CJ83193 virus was detected on Vero cell monolayers.

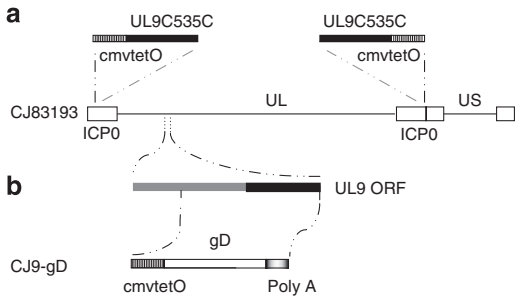

Figure 1. Schematic diagram of genomes of HSV-1 recombinant CJ9-gD. UL and US represent the unique long and unique short regions of the HSV-1 genome, respectively, which are flanked by their corresponding inverted repeat regions (open boxes). (**a**) The replacement of the ICP0 coding sequences with DNA sequences encoding the DNA-binding domain of UL9, UL9-C535C (black box), under control of the tetO-bearing hCMV major immediate-early promoter (vertical line box) in CJ83193 is shown above the diagram of the HSV-1 genome. An expanded UL9 ORF from UL9 locus is shown below the diagram. (**b**) CJ9-gD was generated by replacing the Xcm I-Mlu I fragment encoding UL9 amino acids 217–803 within the UL9 ORF in CJ83193 with the HSV-1 *gD* gene under control of the tetO-bearing hCMV immediate-early promoter. The gradient box shows the poly A signal sequence of bovine growth hormone gene.