Immunization with a Dominant-Negative Recombinant HSV Type 1 Protects against HSV-1 Skin Disease in Guinea Pigs

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CJ9-gD belongs to a new class of replication-defective recombinant herpes simplex viruses (HSVs) type 1 that can function *in trans* to prevent the replication of wild-type HSV in co-infected cells. Furthermore, CJ9-gD cannot establish latent infection *in vivo* and it expresses high levels of the major HSV-1 antigen glycoprotein D immediately following infection. In this study we show that guinea pigs immunized with CJ9-gD developed at least 9,600-fold higher titers of HSV-1-specific neutralization antibodies than mock-immunized controls. After challenge with wild-type HSV-1, all 10 mock-immunized guinea pigs developed multiple skin lesions with an average of 53.3 lesions per animal, whereas only 2 minor lesions were found in 1 of 10 CJ9-gD-immunized animals, representing a 267-fold reduction on the incidence of primary herpetic skin lesions in immunized animals. Quantitative PCR analysis revealed that the amount and frequency of wild-type HSV-1 viral DNA present in dorsal root ganglia of immunized animals was significantly lower than that in mock-immunized controls. Collectively, we demonstrate that vaccination with CJ9-gD elicits strong and protective immune responses against primary HSV-1 skin disease and reduces the extent of latent infection by challenge virus.

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INTRODUCTION

Infection with herpes simplex virus (HSV) type 1 is still a major health issue, as up to 60–90% of people worldwide are seropositive (Whitley and Roizman, 2001; Cowan *et al.*, 2003; Pebody *et al.*, 2004; Cunningham *et al.*, 2006; Xu *et al.*, 2006). Primary infection is followed by establishment of latency in sensory neurons, giving rise to recurrent disease and viral shedding. HSV-1 mainly causes orofacial infections, with 20–40% of the general population experiencing recurrent labial or perioral lesions (Embil *et al.*, 1975). Although outbreaks are usually mild, in immunocompromised individuals, lesions can be more severe and may spread to cause major morbidity (Stanberry *et al.*, 2000). Other cutaneous diseases caused by or associated with HSV-1 include herpetic whitlow, herpes gladiatorum, eczema herpeticum, and erythema multiforme (Hwang and Spruance, 1999). Herpetic keratitis and herpes encephalitis are infrequent but potentially grave complications of orofacial HSV-1 infections (Liesegang, 1991; Whitley, 2006). Moreover, recent reports indicate that, in developed countries, HSV-1 has become the main causative agent for primary genital herpes in adolescents, young women, and homosexual men (Lafferty *et al.*, 2000; Lowhagen *et al.*, 2000; Roberts *et al.*, 2003; Jin *et al.*, 2006).

There is no effective medication to prevent primary infection, no cure for latent infection, and only limited possibilities for treatment and prevention of recurrences (Whitley and Roizman, 2001; Spruance and Kriesel, 2002). Different vaccine strategies have been developed mainly against HSV-2, including peptides, proteins, killed virus, delivering HSV antigens by plasmid DNA and heterologous viral vectors, as well as replication-defective viruses and attenuated replication-competent viruses (Bernstein and Stanberry, 1999; Koelle and Corey, 2003). Subunit vaccines consisting of glycoproteins and an adjuvant appeared to be safe and effective against HSV in several animal models (Stanberry *et al.*, 1987; Ghiasi *et al.*, 1996; Bourne *et al.*, 2005), but showed no or only limited benefit against genital HSV-2 disease in phase III clinical trials (Corey *et al.*, 1999; Stanberry *et al.*, 2002). A live virus vaccine elicits broader and longer-lasting immune responses (Wachsman *et al.*, 2001; Prichard *et al.*, 2005). However, the use of replication-competent viruses raises safety concerns, including the risk of establishing latency and causing morbidity especially in immunocompromised individuals (Meignier *et al.*, 1988, 1990). Replication-defective HSVs lack functions essential for viral replication or assembly of progeny virus particles (Dudek and Knipe, 2006), but elicit protective immune responses in mice and guinea pigs (Nguyen *et al.*, 1992; Boursnell *et al.*, 1997; Brehm *et al.*, 1999; Da Costa *et al.*, 1999).

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Abbreviations: gD, glycoprotein D; HSV, herpes simplex virus

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Nevertheless, replication-defective viruses might regain replication competence in the presence of wild-type virus or induce reactivation of wild-type virus from latent infection. Thus, their use as a vaccine, particularly in latently infected individuals, imposes certain risks.

Using the T-REx (Invitrogen, Carlsbad, CA) tetracycline gene switch technology developed in this laboratory, and the dominant-negative mutant polypeptide, UL9-C5353C, of HSV-1 origin binding protein UL9, we previously generated an UL9-C535C-expressing HSV-1-recombinant CJ83193, capable of blocking its own replication as well as that of wild-type HSV-1 and HSV-2 in co-infected cells (Yao and Eriksson, 1999, 2002). In a mouse ocular model, we demonstrated that CJ83193 is an effective vaccine against wild-type HSV-1 (Augustinova *et al.*, 2004). To further ensure its safety and augment its efficacy as a vaccine against HSV infection, we recently developed a CJ83193-derived HSV-1-recombinant CJ9-gD, which contains an additional deletion in the essential *UL9* gene and an extra copy of the gene encoding the major HSV-1 antigen glycoprotein D (gD) under the control of the tetO-bearing hCMV immediate-early promoter. It is documented that CJ9-gD is completely replication defective, expresses much higher levels of gD than CJ83193 in infected cells, and is significantly more effective than CJ83193 in inducing HSV-1-specific neutralizing antibodies in mice (Lu *et al.*, unpublished data). The ability of CJ9-gD to elicit strong and long-lasting HSV-1 T-cell response has also been illustrated in mice (Brans and Yao, unpublished data).

The guinea pig model of HSV-1 skin infection was established in 1974 by Hubler *et al.*, and since then has been widely used to test various antiviral substances as local treatment against HSV infection (Hubler *et al.*, 1974; Schafer *et al.*, 1977; Alenius and Oberg, 1978; Spruance *et al.*, 1986; Spruance and McKeough, 1988; McKeough and Spruance, 2001). The advantage of the guinea pig model is that the HSV lesions resemble those seen in humans, including development of latency. To our knowledge, this is the first report of using the guinea pig model of skin infection to test the efficacy of a prophylactic vaccine against HSV-1 infection. We demonstrate that immunization with CJ9-gD elicits strong protective immune responses against primary herpes skin disease and reduces frequency and magnitude of latent infection.

RESULTS

Induction of HSV-1-specific IgG and neutralization antibody titers

The average HSV-1-specific IgG antibody titer for guinea pigs immunized with CJ9-gD at a dose of 2×10^7 PFU increased significantly from the second to the third vaccination with a peak value of 6,667 ($P \leqslant 0.05$). The titer did not increase significantly after challenge with wild-type HSV-1 ($P = 0.3$). No HSV-1-specific antibody was detectable by ELISA in a dilution 1:100 of serum from mock-immunized controls (Figure 1a).

The HSV-1-specific neutralization antibody titer was detected in serum from all immunized animals and increased significantly from the second to the third vaccination ($P < 0.05$) with a peak titer 2 weeks after the third vaccination of 19,200. No HSV-1-specific neutralization antibody was detected in serum from mock-immunized animals at 1:2 dilution before challenge. After challenge, the neutralization antibody titer in immunized animals was 2.6-fold higher than that in mock-immunized controls ($P < 0.005$; Figure 1b).

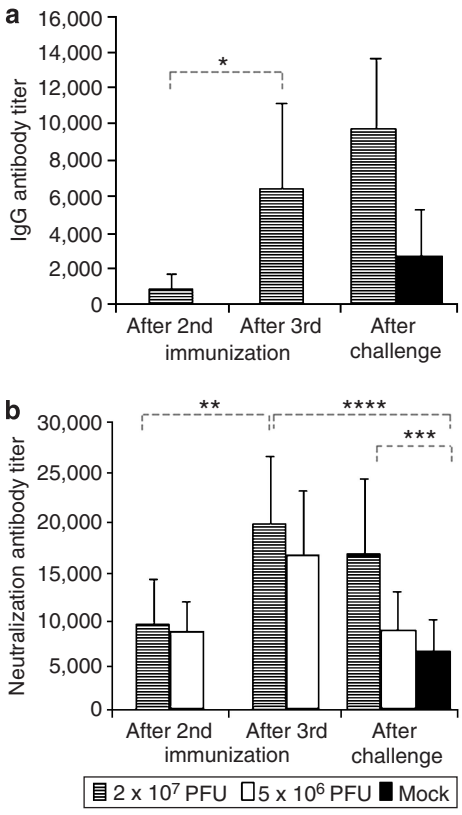

Figure 1. Induction of herpes simplex virus (HSV) type 1-specific IgG and neutralization antibodies. Hartley guinea pigs (two sets of six and one set of eight) were either mock-immunized with DMEM or immunized with CJ9-gD by subcutaneous injection at a dose of 2×10^7 PFU ($n = 6$) or 5×10^6 PFU ($n = 4$) per animal. On days 14 and 28 after primary immunization, guinea pigs were boosted. Serum was obtained from all animals 14 days after each immunization and 28 days after the challenge. (**a**) Unprocessed serum was tested for HSV-1-specific total IgG by ELISA and (**b**) heat-inactivated serum was used for HSV-1-specific neutralization antibody assays. The indicated values represent the mean levels ± SD. *P*-values were assessed by Student's *t*-test (*$P \leqslant 0.05$, **$P < 0.05$, ***$P < 0.005$, ****$P < 0.0005$).

To investigate further the effectiveness of CJ9-gD, the immunization dose was reduced to 5×10^6 PFU per animal, resulting in a neutralization antibody titer of 8,267, which was not significantly lower than those found after immunization with 2×10^7 PFU ($P = 0.5$). Again, the neutralization antibody titer increased after the third immunization (Figure 1b).

Protection from herpetic skin disease

The development and clinical appearance of lesions caused by challenge virus in mock-vaccinated guinea pigs was consistent with the previous observations (Hubler *et al.*, 1974; Alenius and Oberg, 1978). On day 1 after challenge, minor skin irritation due to the viral inoculation was