Rapid Communication

Immunization with a replication-defective herpes simplex virus 2 mutant reduces herpes simplex virus 1 infection and prevents ocular disease

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Abstract

Ocular infections with herpes simplex virus 1 can lead to corneal scarring and blindness, with herpes keratitis being the major infectious cause of blindness. There is currently no clinically approved vaccine and nearly all developmental vaccines are targeted against HSV-2 and genital herpes. We tested the ability of an HSV-2 replication-defective virus, a genital herpes vaccine candidate, to protect against HSV-1 corneal infection. Immunization with HSV-2 dl5-29 reduced viral replication in the cornea, prevented ocular disease and reduced latent infection by the HSV-1 strain. Therefore, this HSV-2 replication-defective mutant strain may have applications for prevention of herpes keratitis and genital herpes due to HSV-1 infection.

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Introduction

Herpes simplex virus (HSV) infects the mucosal epithelium at both orofacial and genital sites. Historically, HSV-1 has been the cause of orofacial infections while HSV-2 has been the agent responsible for genital infections. In a healthy individual, orofacial infections with HSV-1 are usually mild and free from serious complications, resulting in localized vesicular lesions that are resolved within a number of days. However, despite the apparent innocuous nature of these HSV-1 infections, several serious complications are also associated with this virus. HSV-1 infection of the brain results in herpes simplex encephalitis (HSE), while infection of the eye can produce herpetic stromal keratitis (HSK). Both are associated with severe morbidity. There are approximately 300,000 cases of ocular HSV-1 infection diagnosed annually in the US (Roizman et al., 2007).

The initial infection involves the corneal epithelium; however, repeated episodes of recurrent disease can lead to involvement of the underlying stroma. This results in HSK and can eventually lead to blindness (Dawson and Togni, 1976). Generally thought to be a disease with a strong immunopathologic component (Doyzman and Rouse, 1992; Verjans et al., 1998), HSK is the leading cause of infectious corneal blindness in the developed world (Liesegang, 2001). A preventative therapy would be a major public health advance. However, the majority of vaccines currently in development to prevent HSV infection are directed against HSV-2. This is understandable given the view of HSV-2 as a sexually transmitted disease and the emerging evidence that genital herpes, i.e., HSV-2, plays an important role in the acquisition and progression of HIV infection (Corey et al., 2004; Freeman et al., 2006; Nagot et al., 2007; Wald et al., 2002). However, given that serious complications such as HSK and HSE are associated with HSV-1 infection, and the growing epidemiological evidence suggesting an increase in the incidence of genital herpes caused by HSV-1 (Malkin, 2004; Xu et al., 2006), a herpes vaccine should ideally protect against both HSV-1 and HSV-2. Such a vaccine would be capable of preventing the serious outcomes associated with HSV-1 infection, such as HSK, and would also be effective against genital herpes caused by either strain of HSV. With this in mind, we tested the genital herpes vaccine candidate dl5-29, a...
replication-defective HSV-2 virus, for its ability to protect against ocular disease caused by HSV-1 infection. This virus has been shown previously to induce a protective immune response in both murine and guinea pig models of HSV-2 genital infection (Da Costa et al., 1999; Hoshino et al., 2005).

Results

To determine the ability of an HSV-2 replication-defective virus to protect against HSV-1 infection, we immunized mice with the HSV-2 dl5-29 replication-defective mutant virus and later challenged the mice with HSV-1 KOS strain virus via the corneal route, using a corneal infection model described previously (Leib et al., 1989). Mice were immunized subcutaneously on days 0 and 21 with HSV-2 dl5-29 using three different doses, 10⁴ PFU, 10⁵ PFU or 10⁶ PFU. On day 35, these mice and mock-immunized control mice were challenged on the cornea with HSV-1 KOS following scarification. Mice were then monitored for facial lesions, facial swelling and HSV-1 shedding in tear film.

Disease

The control mice that had been mock-immunized with PBS developed both severe facial lesions and pronounced facial swelling following corneal challenge with HSV-1 (Figs. 1 and 2). When compared to these mice, all mice immunized with dl5-29 prior to challenge showed a significant reduction in both lesion severity and facial swelling, regardless of dl5-29 dose. The highest dose of dl5-29 (10⁶ PFU) completely protected the mice from developing facial lesions (Fig. 1) and protected five out of six mice from developing facial swelling (Fig. 2). In the single mouse in this group where swelling was observed, it was mild and transient. The intermediate (10⁵ PFU) and low (10⁴ PFU) doses of dl5-29 provided partial protection against lesion development and facial swelling (Figs. 1 and 2). When compared to the control mice, the reductions in lesion severity seen in the three dl5-29-immunized groups were statistically significant from day 7 through to day 20 post-challenge, with the greatest reductions seen on day 15 post-challenge (p < 0.005 for the 10⁶ PFU group, p < 0.01 for the 10⁵ and 10⁴ PFU groups on day 15). Reductions in facial swelling were statistically significant for the 10⁶ and 10⁵ PFU groups between days 5 and 10 post-challenge, and for the 10⁴ PFU group between days 6 and 8 post-challenge. The greatest reductions in facial swelling were...
in each sample was determined using real-time PCR. Individual TGs were digested and the number of viral genome copies was measured using quantitative PCR. While all mice were found to harbor latent virus within their TGs, we observed a statistically significant (p<0.0005) 10-fold reduction in the number of viral genomes in the TGs of mice immunized with 10^6 PFU of dl5-29 as compared to mock-immunized mice (Fig. 4). As with previous results, mice that had received the intermediate and low dose of dl5-29 showed a reduction in latent viral copies as compared to controls, but the reduction was not as great as seen in those mice that had been immunized with the highest dose of dl5-29, indicating a fairly consistent dose–response.

Discussion

In this study, we observed protection against HSV-1 ocular disease using a systemically administered vaccine directed against HSV-2, to our knowledge the first to do so. An earlier study in a rabbit model of ocular infection used the suboptimal gB2/gD2 subunit vaccine in MF59 emulsion and a live HSV-1 virus vaccine to demonstrate the requirement for periocular vaccination when protecting against HSV-1 ocular disease (Neshburn et al., 1998). The fact that dl5-29 was able to provide such robust protection against ocular disease following systemic administration further supports the evidence that this virus vaccine induces a strong, protective immune response (Da Costa et al., 1999; Hoshino et al., 2005), and provides evidence for the first time that this response is effective against HSV-1. While we did not test the ability of dl5-29 to protect against HSV-1 genital disease, a recent study observed that systemic administration of the HSV-2 subunit vaccine gD2/AS04 could prevent HSV-1 genital disease in a guinea pig model (Bourne et al., 2003). This finding strengthens the idea that a vaccine capable of protecting against genital HSV-2 disease, such as dl5-29 (Da Costa et al., 1999; Hoshino et al., 2005), would also be capable of preventing HSV-1 genital disease.

We have also shown here that dl5-29 is able to reduce the latent viral load in the trigeminal ganglion of immunized mice following HSV-1 challenge. Mice immunized with the highest doses of dl5-29 in this study showed a 10-fold reduction in latent viral load as compared to controls, equivalent to reductions in HSV-2 latent viral load seen with dl5-29 in a guinea pig model of HSV-2 genital infection (Hoshino et al., 2005), further indicating the potential of this virus vaccine against both HSV-1 and -2. Reductions in latent virus may be of particular clinical significance to HSK given the relationship between latent viral loads and reactivation frequencies. Work by Sawtell and others has demonstrated that a reduction in the level of HSV-1 latency correlates with a reduction in the frequency of reactivation (Sawtell, 1998; Sawtell et al., 2001). Given that HSK develops over a series of recrudescent infections, a reduction in reactivation may significantly reduce the development of HSK in immunized individuals. In addi-

**Fig. 4. Levels of latent virus in the trigeminal ganglion are reduced in mice immunized with a replication-defective HSV-2 virus prior to ocular HSV-1 challenge. Groups of 6 to 8 CD-1 male mice were immunized with varying doses of dl5-29 on days 0 and 21 via subcutaneous injection, and a group inoculated with PBS was included as a negative control. On day 35, all mice were challenged with HSV-1 KOS on the eye following corneal scarification. At 1 month post-challenge, the mice were sacrificed, and the trigeminal ganglia were removed. Individual TGs were digested and the number of viral genome copies in each sample was determined using real-time PCR.**
tion, any reduction in reactivation of either HSV-1 or HSV-2 reduces the opportunities for the virus to spread to susceptible individuals.

A vaccine capable of protecting against HSV-2 infection is obviously highly desirable, given the virus’ historical position as the major cause of genital HSV infection and the growing evidence for a link between genital HSV and an increased risk of HIV acquisition, transmission and disease progression (Corey et al., 2004; Freeman et al., 2006; Nagot et al., 2007; Wald et al., 2002). However, a vaccine capable of protecting against both types of HSV would be of even greater public benefit. HSV-1 is the agent responsible for HSK, the leading cause of infectious corneal blindness, and is the most common cause of herpes encephalitis. There is also increasing epidemiological evidence that HSV-1 is becoming a significant cause of genital herpes (Malkin, 2004; Xu et al., 2006), which is likely to lead to an increase in the incidence of neonatal infections caused by HSV-1. The replication-defective virus tested in this study has previously been shown to be effective in preventing genital disease and reducing levels of latent virus in murine and guinea pig models of HSV-2 genital infection. It has also been shown to induce the rapid trafficking of HSV-specific CD8+ T cells to sites of infection following ocular HSV-2 infection in mice (Hoshino et al., 2005). The evidence presented in our current study shows that dl5-29 is able to prevent disease and reduce levels of latency in a mouse model of HSV-1 ocular infection. Combined, these data indicate that dl5-29 could potentially be used as a vaccine to protect against both HSV-1 and HSV-2 infections.

The most obvious caveat to this conclusion is the fact that previous HSV candidate vaccines, most notably the HSV-2 glycoprotein D subunit vaccines, also showed promise in small animal studies while failing to show efficacy in later human trials. However, the subunit vaccines contained only one or two viral glycoproteins and were designed to elicit CD4+ T cell and neutralizing antibody responses (Koelle and Corey, 2003). The replication-defective virus dl5-29 not only provides the immune system with a much more extensive array of viral epitopes but also induces a much broader immune response including CD4, CD8 and antibody responses (Hoshino et al., 2005). Clinical trials are needed to determine how dl5-29 will perform in humans.

Materials and methods

Viruses

The HSV-2 replication defective virus, dl5-29, and wild-type HSV-1 KOS were propagated and assayed for titers on V5-29 (Da Costa et al., 1999) and Vero cells, respectively. The HSV-2 dl5-29 replication-defective mutant virus contains deletions in the U2,5 and U29 genes (Da Costa et al., 1999, 2000).

Mice

CD-1 outbred male mice were obtained from Charles River Laboratories and housed under SPF conditions. All animal protocols were approved by the Institutional Animal Use and Care Committee.

Immunizations and infections

Mice were immunized with varying doses of dl5-29 in PBS or with PBS alone via subcutaneous injection in a total volume of 100 μl. Mice were immunized on days 0 and 21 and then infected on the cornea on day 35. For the corneal infection (Leib et al., 1989), mice were anesthetized with 13 μl/g ketamine (80 mg/ml)/xylazil (12 mg/ml) (Sigma) via intraperitoneal injection. A 30-gauge needle was used to scratch the eye 20 times, 10 times in a vertical direction and 10 times in a horizontal direction. A 5 μl aliquot of HSV-1 KOS containing 2 × 10⁶ PFU was placed on the eye and allowed to adsorb before the mice regained consciousness.

Viral shedding

On days 1 to 7 post-challenge, mice were anesthetized briefly with Isoflurane (Abbott Labs); sterile cotton applicators (Puritan Medical Products) soaked in Dulbecco’s minimum essential medium (DMEM) (Invitrogen) were used to collect the fluid on each eye. For each mouse, the samples from both eyes were placed together in a 3 ml glass vial containing 1 ml sterile DMEM and stored at −80 °C until needed. To determine viral shedding, plaque-forming assays were performed whereby serial dilutions of the medium in each vial were plated onto confluent Vero cell monolayers (Morrison and Knipe, 1994). Statistical analysis was performed using the unpaired Student’s t-test.

Facial lesions and swelling

Between days 5 and 15 post-challenge, mice were monitored for facial swelling. Facial swelling scores were based on the presence of swelling in 3 different regions of the left and right sides of the face. Swelling in each individual area earned a score of 1, with a maximum score of 6 possible. Mice were monitored for facial lesions between day 6, when lesions first appeared, and day 20 post-challenge. Lesions were scored based on the severity of lesions on the left and right sides of the face. Each side was scored from 0 to 4, with 0 indicating no lesions and 4 indicating severe lesions. The scores from either side were then added together, giving a maximum possible score of 8.

Quantitative PCR

On day 30 post-ocular challenge, the TGs were removed from each mouse and immediately placed on dry ice before being transferred to −80 °C until required. Each TG was digested overnight at 56 °C in protease K digestion buffer (200 μg/ml protease K, 25 mM KCl, 5 mM Tris, pH 9, 500 μM MgCl₂). The exact volume of each sample was then determined, and 1 μl of each sample was used in a real-time PCR reaction. Real-time PCR amplification and detection were performed using an ABI 770 Sequence Detector, with HSV-1 thymidine kinase gene specific primers used to detect HSV-1.
KOS viral DNA: Forward primer: ACCCGCTTAACAGCCTCAACA, Reverse primer: CCAAGAGGCTGGGGAGTTT (Katz et al., 1990; McKnight, 1980). Reactions were performed in 10 μl volumes containing SYBRGreen PCR mix and primers at a final concentration of 500 nM. PCR reactions were amplified at 50 °C for 2 min, 95 °C for 10 min, then 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

In each reaction, a standard curve was included. Total genomic DNA was extracted from HSV-1 infected Vero cells using the QIAGEN DNeasy tissue kit. Viral DNA was obtained by running the total genomic DNA on a 0.9% Low Melt agarose gel and extracting the viral band. The viral DNA was then quantified, using absorbance and comparison to mass standards on a gel, to determine the number of viral genomes/μl. To create a standard curve, the purified HSV-1 KOS DNA was serially diluted such that each reaction contained 10-fold dilutions of HSV-1 DNA from 2 × 10^7 to 2 × 10^0 molecules.

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References


Malkin, J.E., 2004. Epidemiology of genital herpes simplex virus infection in developed countries. Herpes 11, 2A–23A.


Sawtell, N.M., 1998. The probability of in vivo reactivation of herpes simplex virus type 1 increases with the number of latently infected neurons in the ganglia. J. Virol. 72, 6888–6892.


