Ocular Safety and Efficacy of an HSV-1 gD Vaccine During Primary and Latent Infection

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One potential complication of systemic herpes simplex virus (HSV) vaccination is that subsequent ocular infection may lead to increased immunogenic corneal scarring. Therefore, V52, a genetically engineered vaccinia virus that expresses the HSV-1 glycoprotein gD, was tested for ocular safety and for protection against ocular challenge with a stromal-disease-producing strain (McKrae) of HSV-1. To maximize immune response, rabbits were vaccinated by a series of inoculations. V52-vaccinated rabbits developed significant HSV-1 neutralizing antibody titers; however, they were not as high as those induced by vaccination with live HSV-1 McKrae. One month after the final vaccination, all rabbits were challenged ocularly. Eyes were monitored for 35 days for epithelial keratitis, stromal keratitis, and iritis. In no case was epithelial keratitis, stromal keratitis, or iritis significantly exacerbated by vaccination. The gD V52 recombinant vaccine provided protection against HSV-1 induced epithelial keratitis ($P = 0.02$) and long-term stromal scarring ($P = 0.04$). There was no significant reduction in the incidence of trigeminal ganglionic latency in the vaccinated rabbits ($P > 0.05$). Thus, our results indicate that V52, a gD recombinant vaccine probably is safe with regard to corneal scarring, and may provide a small amount of protection against ocular HSV-1 infection. The amount of protection provided was less than that reported in mice and guinea pigs. This suggests that to provide high levels of ocular protection in rabbits (and probably in humans), HSV-1 vaccines may have to elicit a more vigorous immune response than that produced by normal HSV-1 infection. Invest Ophthalmol Vis Sci 31:1497-1502, 1990

Herpes simplex virus (HSV) is the leading cause of viral-induced blindness in man. Corneal inflammation and scarring produced by HSV appears to be caused by an as yet undefined host immune reaction to viral antigen(s).

The host immune response to HSV-1 is directed mainly at the viral glycoproteins. The HSV-1 glycoprotein genes gB and gD have been incorporated into vaccinia virus and shown to induce some immunity in vaccinated animals; however, their ocular safety was not examined.

Modifying the host immunologic status by systemic HSV vaccine could change the host response to subsequent eye infection with a stromal-disease-producing strain of HSV. Systemic HSV vaccination might ameliorate ocular infection or it could exacerbate the immunologic process that produces corneal inflammation and scarring. Therefore, in addition to evaluating their possible efficacy in ameliorating HSV-induced stromal keratitis and their effect on latency and reactivation, all candidate vaccines must be tested for their ocular safety.

To evaluate ocular safety and possible efficacy, we chose an ocular rabbit model of HSV-1 infection, since it closely resembles the human disease. Animals were immunized intradermally with either the vaccinia–HSV gD recombinant candidate vaccine (V52), live McKrae HSV-1 as the positive control, or V36, a vaccinia–influenza hemagglutination (HA) recombinant as the negative control. Rabbits were challenged by ocular infection with a stromal-disease-producing strain of HSV-1 (McKrae) 1 month after systemic vaccination was completed. The effect of vaccination on ocular HSV challenge and subsequent induced reactivation was evaluated in a masked fashion.
Materials and Methods

Virus and Vaccines

McKrae, a stromal-disease-causing HSV-1 strain, was used as the positive control vaccine and as the challenge virus. The candidate anti-HSV-1 vaccine, designated V52, was obtained from B. Moss. This genetically engineered recombinant vaccinia virus strain expresses the HSV-1 glycoprotein gD under control of the vaccinia late promoter. V36, used as a negative control, is an identical vaccinia recombinant, except that it expresses the unrelated influenza HA glycoprotein in place of the HSV-1 gD glycoprotein.

Rabbits

New Zealand White male rabbits (approximately 2 kg each) (Universal Animal Care) were used for all experiments. These animals develop a primary and recurrent herpetic disease that mimics HSV keratitis in man. Animal care and treatment in this investigation were in compliance with the ARVO Resolution on the Use of Animals in Research.

Vaccination Protocol

Each of thirteen rabbits in each group was vaccinated intradermally at five different locations on its shaved back with a total of 10^8 viable pfu of either V52 (gD) (experimental group), V36 (negative control), or HSV-1 McKrae (positive control). All rabbits were revaccinated 3 weeks later with the same protocol.

Virus Challenge

Rabbits were challenged ocularly 1 month after the second vaccination. All rabbits were bilaterally infected without corneal scarification by placing approximately 1-2 X 10^5 pfu of virus into the conjunctival cul-de-sac, closing the eye, and rubbing gently for 30 sec.

Serum Neutralizing Antibody Titers

Serum neutralizing antibody titers were determined by plaque reduction assays. Briefly, 2-fold serial dilutions of heat-inactivated rabbit serum were made with the use of tissue culture medium containing 10% fetal calf serum (GIBCO, Gaithersburg, MD) as the diluent. Each dilution was incubated with an equal volume of HSV-1 McKrae virus suspension for 30 min. Aliquots containing approximately 100 pfu prior to neutralization were assayed for plaque production on CV-1 cells under agar. Neutralizing antibody titers are expressed as the highest serum dilution resulting in a reduction of at least 50% in plaques.

Ocular Parameters

The presence of ocular infection was monitored by tear film cultures. Severity of ocular involvement was scored on a 0 to 4 scale in a masked fashion by examination with slit-lamp biomicroscopy with 1% methylene blue (Sigma, St. Louis, MO) to delineate epithelial ulceration. Iritis and stromal keratitis were scored similarly.

In Vivo Reactivation

In vivo reactivation was done by iontophoresis of 6 hydroxydopamine (6HD) (Sigma) followed by application of topical epinephrine (Allergen, Hormigueros, Puerto Rico). An eyecup was inserted under the lid of the anesthetized rabbit so that it made contact with the corneal limbus. A freshly prepared 0.1% 6HD solution was added to the eyecup, and a direct current of 0.5 mA was applied for 8 min. One drop of 2% 1-epinephrine hydrochloride was administered immediately and then twice daily for 4 days. This method yields positive eye cultures in approximately 90% of rabbits latently infected with McKrae. Eyes were sampled for HSV-1 by tear film cultures for up to 10 days after iontophoresis.

Statistical Analysis

The Mann-Whitney Rank Sum test was used for the statistical analysis of serum neutralizing titers, and of peak levels of epithelial keratitis, stromal keratitis, and iritis. The student t-test was used to compute the significance of long-term stromal scarring.

Results

In pilot experiments, 13 rabbits were vaccinated intradermally with a single inoculum containing 10^7 pfu of live V52 recombinant virus. The results were disappointing. The rabbits developed HSV-1 neutralizing antibody titers that were not significantly higher than the mock-vaccinated negative control animals. After ocular challenge with a moderate-to-high dose of HSV-1 strain McKrae (2 x 10^5 pfu/eye), no significant differences were detected between the experimental group and the unvaccinated control group for any of the ocular parameters tested (data not shown).

To increase the immune response to the V52 vaccine, we altered our inoculation regimen. In the vaccine trial reported here, each rabbit was inoculated intradermally at five different locations on its back, with a total of 10^9 pfu of V52 (ten times more V52 than in the first trial) per rabbit. To further maximize...
the immunologic response, vaccination was repeated 3 weeks later. Negative controls received $10^8$ pfu of the vaccinia recombinant V36, according to the same regimen. Positive controls received $10^8$ pfu of live challenge virus, HSV-1 McKrae, also according to the same regimen.

**Prechallenge Immune Responses**

After the first vaccination, all rabbits in all three groups developed typical skin lesions lasting 7–12 days. These lesions indicated that the biologic response to the local skin infection caused by the growth of the vaccinia virus recombinants (V52, V36) or HSV-1 (McKrae) had occurred. After the second vaccination, approximately half of the animals in each group developed no lesions. The remaining animals developed only minimal viral-induced lesions, which lasted less than 5 days. This suggested that the animals had developed some immune response to the vaccinia virus recombinants and to the HSV-1 virus with which they had been vaccinated.

Four weeks after the second inoculation, the rabbits were bled and the sera tested for HSV-1 neutralizing titers by plaque reduction assays. Neutralizing antibody titers are expressed as the highest serum dilution resulting in a reduction of at least 50% in plaques. Rabbits vaccinated with the V52 vaccine had average neutralizing titers of approximately 1:20. This was significantly greater ($P = 0.001$) than the negative control (V36) rabbits, which had an average titer of less than 1:1. The positive control rabbits vaccinated with live HSV-1 had an average titer of approximately 1:116, significantly higher than the V52 rabbits ($P = 0.008$). Thus, although the recombinant vaccine (V52) was capable of inducing HSV-1 serum neutralizing titers, the titers were not as high as those induced by inoculation with live HSV-1. This may be because of the ability of live HSV-1 to induce antibodies to viral proteins in addition to gD; because of a poorer response to gD with V52; or because of a combination of these two possibilities.

**Ocular Challenge**

One month after the final (second) vaccination, all rabbits were ocularly challenged with HSV-1 (McKrae), at $2 \times 10^3$ pfu/eye. Tear film cultures were collected between days 2 and 10 and tested for the presence of HSV-1. All eyes in the vaccinated and control groups shed HSV-1 at some point during this period. Tear film virus was not titred, but there was no difference in the incidence of acute external ocular viral infection among any of the test groups.

**Epithelial Keratitis**

As indicated in Figure 1A, peak epithelial keratitis occurred on day 7 for all vaccinated groups. Both the V52 group and the HSV-1 positive control group ap-
peared to show slightly less epithelial involvement than did the control group on this day. Analysis of the data for day 7 indicated that the slight protection observed was statistically significant \((P = 0.02\) for the V52 group and \(0.008\) for the HSV-1 positive control group). Thus, the V52 vaccine conferred a small amount of protection against epithelial keratitis in rabbits challenged with HSV-1 McKrae.

**Stromal Keratitis**

The early transient or disciform edema phase of stromal keratitis peaked on day 7 for all groups (Fig. 1B). Rabbits vaccinated with McKrae HSV-1 showed slight but statistically significant protection from early stromal involvement on day 7 \((P = 0.002\). However, no statistically significant protection was provided by V52 vaccination \((P = 0.17\).

At 24–35 days after challenge there appeared to be a difference in keratitis-induced stromal scarring between the V36 (negative control) rabbits and the V52- and HSV-1-vaccinated rabbits. This was more apparent when the data for day 35 were presented in a different form (Fig. 2). Three of 18 eyes from the V36-vaccinated rabbits showed severe (4+) stromal scarring. In contrast, none of the eyes from either V52-vaccinated rabbits or HSV-1-vaccinated rabbits showed more than mild (1+) stromal scarring. The differences between long-term stromal scarring in vaccinated and nonvaccinated rabbits were significant at a 0.05% probability level according to the student t-test (V52 vs V36, \(P = 0.04\); HSV-1 vs V36, \(P = 0.03\)). Thus, V52 vaccination may have provided some protection against long-term stromal scarring. Comparison of pooled data from the V52- and HSV-1-vaccinated groups compared to the V36 control group yielded a \(P\) value of 0.003, indicating protection against long-term clouding at a 0.01 probability level.

**Iritis**

As with epithelial keratitis and stromal keratitis, the peak iritis occurred on day 7. Rabbits vaccinated with either the V52 recombinant or live HSV-1 were not protected from iritis (Figure 1C; \(P = 0.20\) and \(P = 0.54\), respectively). Although rabbits vaccinated with V52 appeared to have slightly more iritis than did the negative control (V36), this difference was not statistically significant.

**Postchallenge Serum Titers**

Fifty days after ocular challenge, rabbits were bled and serum neutralization titers were determined. The response of the negative control V36-vaccinated rabbits to McKrae HSV-1 ocular challenge resulted in an average titer of 1:80. The V52-vaccinated and the positive control HSV-1-vaccinated rabbits attained average postchallenge titers of 1:165 and 1:171 respectively. These titers were significantly higher than the V36 control titers \((P < 0.03)\) and similar to each other \((P = 0.86)\). Thus, vaccination with either the V52 recombinant or HSV-1 resulted in a similar enhanced immune response after ocular challenge.

**In Vivo Iontophoresis-Induced Reactivation**

To determine the effect of vaccination on latency, iontophoresis of 6HD followed by application of topical epinephrine\(^3\) was performed between 45 and 50 days postchallenge. Eyes were sampled for HSV-1 by tear film cultures for up to 10 days after iontophoresis. Eighty-nine percent (8 of 9) of the V36-vaccinated rabbits could be reactivated. This result is similar to the usual in vivo inducible reactivation rate for rabbits latently infected with McKrae. In contrast, only 55% (6 of 11) of the V52-vaccinated rabbits and 63% (5 of 8) of the HSV-1-vaccinated rabbits could be induced to reactivate. These reductions in reactivation ability suggest a partially protective effect due to vaccination. However, they were not statistically significant \((P = 0.12)\).

**Discussion**

The goals of this study were to determine the ocular safety and efficacy of systemic vaccination with
the vaccinia-HSV-1 gD recombinant vaccine V52, after ocular challenge with a stromal disease producing strain of HSV-1. None of the ocular parameters examined was exacerbated by vaccination with either V52, or live, homologous, McKrae strain HSV-1. These results in rabbits indicate that this candidate systemic vaccine did not exacerbate experimentally induced keratitis, scarring, or iritis.

Analysis of the ocular efficacy of systemic vaccination with V52 (and high levels of homologous live HSV-1) was more complicated. Our results indicated that in rabbits, systemic vaccination with either the V52 recombinant vaccine or with live McKrae HSV-1 provided a small amount of protection against epithelial keratitis and long-term stromal keratitis (scarring) after subsequent high-titer HSV-1 ocular challenge 1 month postvaccination. In addition, vaccination with HSV-1 also provided some protection against transient disciform stromal keratitis. Since ocular challenge was not carried out at any other time, it is not known whether this subtle protection afforded by systemic HSV-1 vaccination is long-lasting.

Although the protection afforded against ocular challenge in rabbits was statistically significant, the absolute amount of protection was small (Figs. 1, 2). In contrast, in ocular and nonocular mouse models, vaccines using vaccinia-expressed gD, virion- or recombinant-purified gD, and synthetic gD related peptides provided higher levels of protection against primary infection and up to 70% protection against establishment of latency.12-16 Protection against primary and latent genital infections have also been achieved in guinea pigs.15,17,18

The reason for the inability to achieve high levels of protection against ocular challenge in the rabbit, even with high titers of HSV-1 as a systemic vaccine, is not known. We postulate that this difference is due to either 1) a fundamental difference in some phase of the immune response in rabbits compared to mice, such as local ocular immunity or cell-mediated immunity, which we did not measure in this study, but which may play a role in protection from herpetic disease;19 or 2) increased isolation or compartmentalization of the rabbit eye from the immune system compared to mice, perhaps due to increased relative corneal diameter and isolation of the cornea from the limbal blood supply.

If the difference is due to one of these factors, it will be important to determine whether the mouse or rabbit model more closely reflects the human situation. In general, the rabbit has been a better model for human ocular HSV-1 infection than has the mouse. Certainly, if partial immune isolation due to corneal size is a major factor, then the rabbit model will be more relevant. In this case, it may prove very difficult to protect against human ocular HSV-1 infection by systemic vaccination. If the reduced ocular vaccine efficacy is due to reduced immune response in the eye (either because of systemic or local differences), it may be necessary for future candidate HSV vaccines to generate a much more intense immune response than is induced by natural infection, in order to provide ocular protection.

**Key words:** herpes simplex virus, vaccine, safety, infection, latency

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**References**

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