

SHORT REPORT

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## Herpes simplex virus type 2 trivalent protein vaccine containing glycoproteins C, D and E protects guinea pigs against HSV-1 genital infection

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### ABSTRACT

A vaccine to prevent genital herpes is an unmet public health need. We previously reported that a trivalent vaccine containing herpes simplex virus type 2 (HSV-2) glycoproteins C, D, and E (gC2, gD2, gE2) produced in baculovirus and administered with CpG/alum as adjuvants blocks immune evasion mediated by gC2 and gE2 and virus entry by gD2. The vaccine protected guinea pigs against HSV-2 vaginal infection. We evaluated whether the HSV-2 vaccine cross-protects against HSV-1 because many first-time genital herpes infections are now caused by HSV-1. Guinea pigs were mock immunized or immunized with the trivalent vaccine and challenged intravaginally with a different HSV-1 isolate in two experiments. Guinea pigs immunized with the trivalent vaccine developed genital lesions on fewer days than the mock group: 2/477 (0.4%) days compared to 15/424 (3.5%) in experiment one, and 0/135 days compared to 17/135 (12.6%) in experiment two (both  $P < .001$ ). No animal in the trivalent group had HSV-2 DNA detected in vaginal secretions: 0/180 days for trivalent compared to 4/160 (2.5%) for mock ( $P < .05$ ) in experiment one, and 0/65 days for trivalent compared to 4/65 (6%) for mock in experiment two. Therefore, a vaccine designed to prevent HSV-2 also protects against HSV-1 genital infection.

### ARTICLE HISTORY

Received 3 February 2020  
Revised 10 March 2020  
Accepted 26 March 2020

### KEYWORDS

Prophylactic genital herpes vaccine; HSV-1; HSV-2; trivalent protein vaccine; guinea pigs; glycoprotein C; glycoprotein D; glycoprotein E; immune evasion

A vaccine that prevents genital herpes infection has important public health implications. People with genital herpes have a 3-fold higher risk of acquiring and transmitting HIV.<sup>1</sup> Infants delivered through birth canals infected with herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) are at risk for disseminated infection that may result in severe neurologic impairment or death.<sup>2</sup> Some individuals with genital herpes have painful genital lesions during first-time and/or recurrent outbreaks, while others have only asymptomatic infection and are unaware of their infection status. Nevertheless, both symptomatic and asymptomatic individuals risk transmitting virus to their intimate partners.<sup>3</sup>



The rate of genital herpes infection caused by HSV-2 is declining, while HSV-1 rates have remained stable since 1993.<sup>4</sup> Approximately 50% of first-time genital herpes infections in resource-rich countries are now caused by HSV-1.<sup>5</sup> However, HSV-2 remains the most frequent cause of genital outbreaks, in part because HSV-2 is more likely to recur than HSV-1.<sup>5–7</sup> Therefore, a vaccine that protects against both HSV-1 and HSV-2 genital herpes is necessary.

Vaccines intended to prevent HSV-2 genital herpes have thus far been unsuccessful.<sup>8–10</sup> One such vaccine was evaluated in the Herpevac Trial for Women that enrolled HSV-1 and HSV-2 seronegative women (uninfected women) who were immunized with an entry molecule, gD2, administered with MPL and alum as adjuvants.<sup>10</sup> The vaccine did not prevent HSV-2 genital disease, yet the vaccine was efficacious against HSV-1 genital disease.<sup>10</sup> A subset analysis of 30 sera from gD2-vaccinated women in the Herpevac Trial demonstrated 3.5-fold higher

serum neutralizing antibody titers to HSV-1 than HSV-2 as a possible explanation for the cross-protection.<sup>11</sup> Studies in cotton rats and guinea pigs reported that HSV-2 immunization protects against HSV-1 genital infection, providing supporting evidence for the Herpevac Trial results.<sup>12,13</sup> Additional support for cross-protection afforded by HSV-2 immunity comes from studies that reported HSV-2 infection prior to pregnancy protected against HSV-1 genital infection during pregnancy.<sup>14</sup>

We are evaluating a prophylactic HSV-2 glycoprotein vaccine for genital herpes.<sup>15,16</sup> The vaccine is based on the hypothesis that HSV immune evasion may contribute to vaccine failure. Glycoprotein C (gC) binds complement component C3b to inhibit complement activation.<sup>17–19</sup> Glycoprotein E (gE) functions as an IgG Fc receptor to inhibit activities mediated by the IgG Fc domain, including complement activation and antibody dependent cellular cytotoxicity.<sup>20,21</sup> Our vaccine candidate contains HSV-2 gC2/gD2/gE2 antigens that produce antibodies to block virus entry mediated by gD2 and immune evasion from antibody and complement mediated by gC2 and gE2.<sup>22–24</sup> The gC2/gD2/gE2 trivalent protein vaccine is highly efficacious in protecting mice and guinea pigs from genital lesions and death after HSV-2 intravaginal challenge and is significantly more effective than gD2 alone.<sup>15,25</sup>

We previously reported that serum from mice immunized with the trivalent protein vaccine neutralizes HSV-1 as effectively as HSV-2, suggesting that the vaccine may cross-protect against intravaginal HSV-1 infection.<sup>16</sup> We evaluated for cross-protection by immunizing Hartley strain female guinea pigs three times at two-week intervals with the trivalent HSV-2

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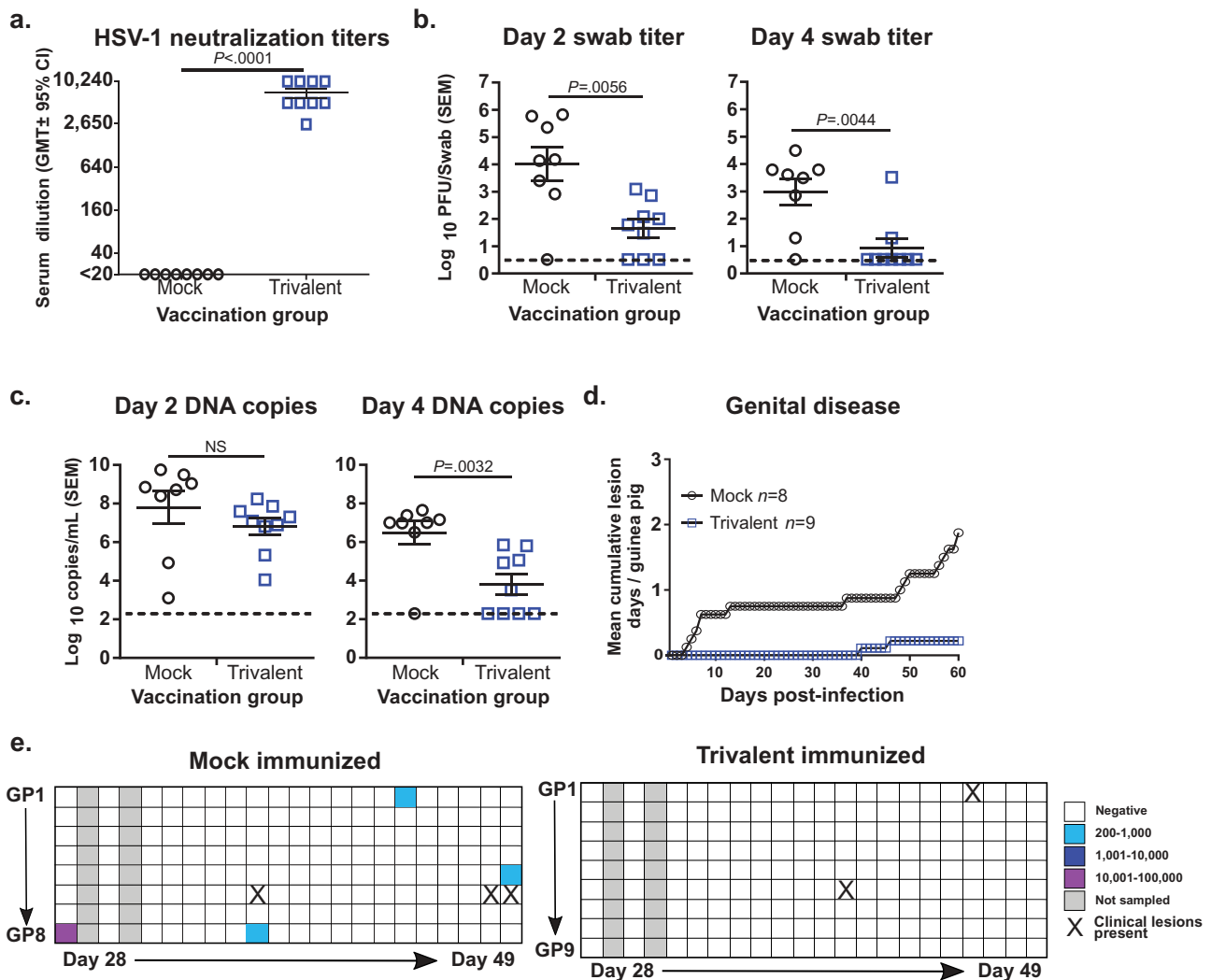
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gC2/gD2/gE2 protein vaccine administered with CpG and alum as adjuvants ( $n = 9$ ) or mock immunized with CpG and alum alone ( $n = 8$ ). Two weeks after the final immunization, serum was obtained for neutralizing antibody titers (Figure 1a). The mean HSV-1 serum neutralizing antibody titer in gC2/gD2/gE2-immunized animals was 1:7,111 compared to <1:20 (negative) in mock-immunized animals. The HSV-1 neutralizing antibody titers are comparable to those we previously reported against HSV-2 in gC2/gD2/gE2 immunized guinea pigs.<sup>15</sup>

Animals were infected intravaginally with  $1 \times 10^6$  PFU of HSV-1 NS, a low passage clinical isolate.<sup>27</sup> The HSV-1 NS dose for guinea pigs was chosen, in part based on our prior

study in mice that reported vaginal disease in 5/5 (100%) mice and death in 3/5 (60%) mice infected intravaginally with a 10-fold lower dose of HSV-1 NS.<sup>28</sup> Vaginal swabs were obtained on days two and four post-infection and assessed for virus titers by plaque assay and HSV-1 DNA copy number by qPCR.<sup>16</sup> HSV-1 virus titers were significantly reduced in the vaginal fluids of gC2/gD2/gE2-immunized animals on days two and four compared to mock-immunized animals (Figure 1b). Despite significantly lower virus titers in the trivalent group, the HSV-1 DNA copy number on day two was not significantly reduced compared with mock-immunized animals (Figure 1c). We postulate that much of the virus in the



**Figure 1.** Trivalent gC2/gD2/gE2 subunit protein immunization protects guinea pigs against HSV-1 NS intravaginal infection. Female Harley strain guinea pigs were immunized intramuscularly with 10  $\mu$ g each of gC2, gD2, and gE2 subunit proteins administered with 100  $\mu$ g of CpG and 150  $\mu$ g of alum as adjuvants ( $n = 9$ ). Animals were immunized three times at two-week intervals. Mock-immunized guinea pigs were vaccinated with CpG/alum without HSV-2 glycoproteins ( $n = 8$ ).<sup>15</sup> Two weeks after the final immunization, serum was collected followed by intravaginal infection with  $1 \times 10^6$  PFU of HSV-1 strain NS. (A) Serum neutralizing antibody titers were determined and are reported as the serum dilution that reduces the number of plaques by 50% in the presence of 10% HSV-1/HSV-2 seronegative human complement.<sup>24</sup> (B) Vaginal swabs were obtained for virus titers two days and four days post-infection. The swabs were placed in 1 mL of DMEM containing 5% fetal bovine serum supplemented with vancomycin (25  $\mu$ g/mL). Serial 10-fold dilutions of the swab media were added to Vero cells and viral titers determined by plaque assay.<sup>26</sup> The dashed line indicates the limit of detection of 3.3 PFU/mL. (C) Viral DNA was isolated from the same swabs as in “B” using the Qiagen DNeasy Blood and Tissue Kits according to the manufacturer’s instructions. Viral DNA was detected by qPCR using 5  $\mu$ L of sample DNA. The HSV-1 copy number was determined based on a standard curve using purified HSV-1 DNA obtained from the American Type Culture Collection.<sup>16</sup> The limit of detection of the assay is one copy of HSV-1 DNA in 5  $\mu$ L, which is equivalent to 200 copies/mL (indicated by the dashed line). (D) Guinea pigs were evaluated for genital disease for 53 days between days 1–60 post-infection and the cumulative lesion scores plotted. (E) Swabs were obtained for vaginal shedding of HSV-1 DNA for 20 days between days 28–49 post-infection. The gray shaded columns represent days vaginal swabs were not collected (days 29 and 31) DNA from the vaginal swab samples was purified using a Qiagen column, collected in 200  $\mu$ L, and 5  $\mu$ L amplified by qPCR. Samples with less than one copy by 40 cycles were considered negative, while positive samples were reported as HSV-1 DNA copies per mL (limit of detection 200 copies/mL).<sup>16</sup>

trivalent group was either degraded by the immune response or coated with neutralizing antibodies rendering it noninfectious to account for the high copy number of HSV-1 DNA but low virus titers.

The guinea pigs were observed daily for genital lesions on 53 days between 1–60 days post-infection. Two animals of nine in the trivalent group had genital lesions; each animal had lesions on only one day for a total of two days with genital lesion on 477 (0.4%) observation days. By comparison, four of eight animals in the mock group had genital lesions on 15 of 424 (3.5%) observation days ( $P = .0008$  comparing lesion days, Fisher's Exact test) (Table 1). Mean cumulative lesions days per guinea pig in the trivalent and mock groups are shown in Figure 1d. Genital swabs were processed for HSV-1 DNA copy number by qPCR to detect recurrent genital infection on 20 days between days 28–49 post-infection. None of nine trivalent protein immunized animals had HSV-1 DNA detected in vaginal secretions sampled on 180 days (0/180, 0%) compared to three of eight guinea pigs in the mock immunized group that shed HSV-1 DNA on 4 of 160 (2.5%) days ( $P = .0481$  comparing days of vaginal HSV-1 DNA shedding, Fisher's Exact test) (Table 1) (Figure 1e). No animal in either group had HSV-1 DNA detected in the dorsal root ganglia (DRG) at the end of the experiment. The absence of HSV-1 DNA in DRG is consistent with our prior guinea pig results that suggested vaginal shedding of HSV DNA during the recurrent phase of infection is a more sensitive marker for latent infection than HSV DNA in DRG.<sup>16</sup>

We evaluated whether the trivalent protein vaccine provides protection against HSV-1 17syn<sup>+</sup>, which is a laboratory-adapted strain. Our prior studies indicated that HSV-1 strains NS and 17syn<sup>+</sup> produce comparable disease in the mouse flank model.<sup>28</sup> Guinea pigs were immunized with the trivalent vaccine ( $n = 5$ ) or mock immunized ( $n = 5$ ) and challenged with  $5 \times 10^5$  PFU of HSV-1 17syn<sup>+</sup>. The challenge dose of  $5 \times 10^5$  PFU was selected, in part based on a report that used this dose to challenge guinea pigs immunized with an experimental gD2 MPL/alum (ASO4) vaccine.<sup>13</sup> Vaginal swabs were obtained for virus titers and HSV-1 DNA copy number on days two and four post-infection. Virus titers were significantly lower in the trivalent than mock-immunized animals on day two, while on day four the titers were lower in the trivalent group, although differences were not statistically significant ( $n = 5$ ) (Figure 2a). Similar to results shown in Figure 1c, HSV-1 DNA copy number was not reduced on day two in the trivalent group (Figure 2b) compared to mock animals despite the significantly lower virus titers in the

trivalent group. These results again support our hypothesis that the virus was either degraded by vaccine-induced immunity or coated with neutralizing antibodies to render it noninfectious.

The guinea pigs were observed for genital lesions for 27 days (Figure 2c). None of five animals in the trivalent protein group developed genital lesions (total of 135 observation days) compared to three of five animals in the mock group that had genital lesions on 17/135 (12.6%) days ( $P < .0001$  comparing lesion days, Fisher's Exact test) (Table 1). Vaginal swabs were evaluated for HSV-1 DNA copy number between days 15–27. The five trivalent-immunized animals had no shedding of HSV-1 DNA during the 13-day sampling period (0/65 days), compared with two of five guinea pigs that shed HSV-1 DNA on 4/65 (6%) days ( $P = .1192$  comparing shedding days) (Table 1) (Figure 2d). Dorsal root ganglia harvested at the end of the experiment were positive for HSV-1 DNA in one of five animals in the mock group and none of five in the trivalent group.

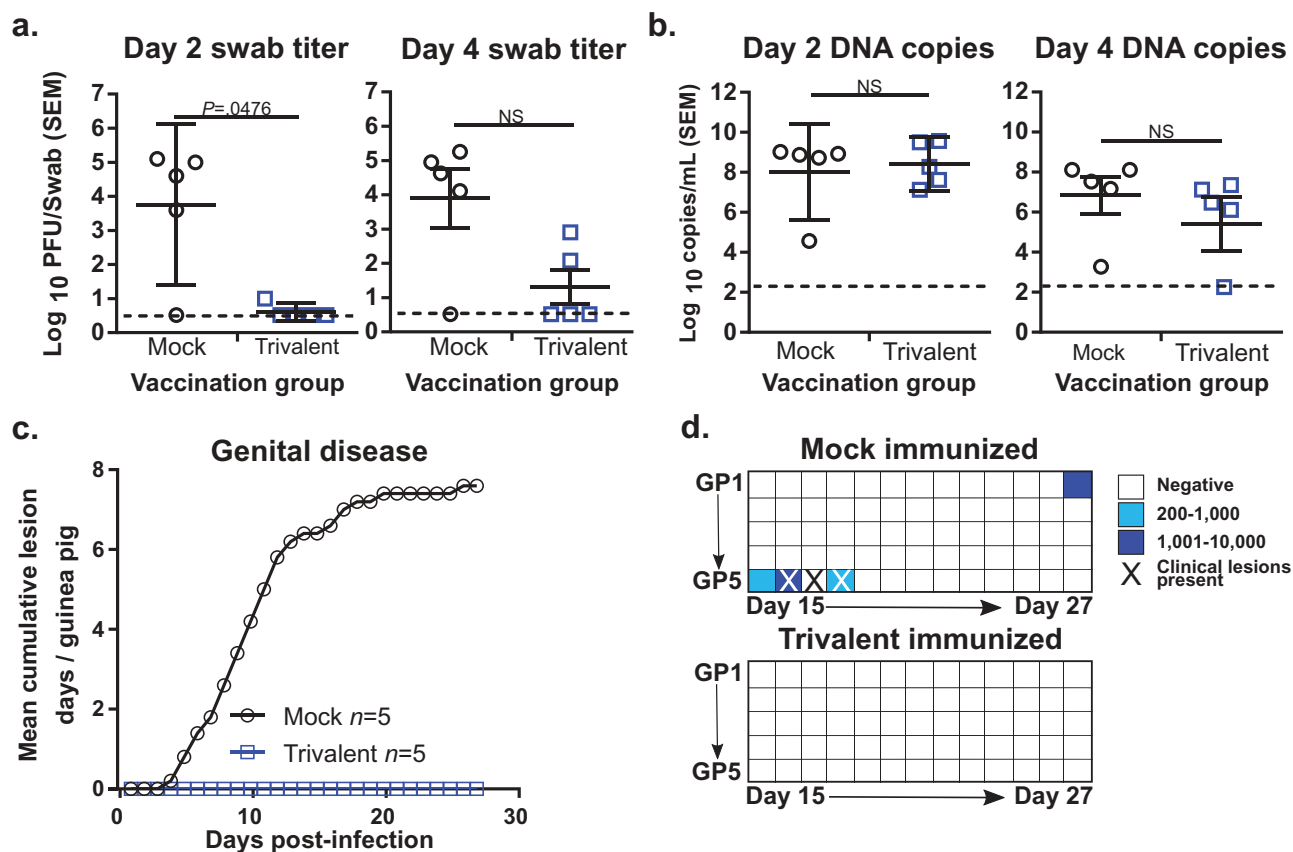
This study has several limitations. 1) Both experiments had a relatively small sample size with 17 guinea pigs (8 or 9 per group) in one study and 10 (five per group) in the other. We used two different HSV-1 isolates for the challenge studies. The decision to evaluate different strains does not permit us to combine the results from the two experiments to improve the power of our results; however, it does make our vaccine results more generalizable in that NS is a low-passage clinical isolate and 17syn<sup>+</sup> is a laboratory-adapted strain. 2) Animals were followed for genital lesions over 60 days in one experiment and 27 days in the other. Despite the small sample size and varying days of observation, we detected highly significant differences between mock and trivalent immunized animals for days with genital lesion in both experiments. 3) Vaginal shedding of HSV-1 DNA was monitored over different time periods post-infection. In one study, vaginal shedding of HSV-1 DNA was evaluated for 20 days between days 28–49, while in the other, HSV-1 DNA shedding was assessed for 13 days between days 15–27. Although not symmetrical, an advantage of the study design was that we were able to assess vaccine efficacy over 33 days at both early and later times post-infection.

We propose two hypotheses that are not mutually exclusive for why HSV-2 immunogens protect against genital HSV-1 infection. First, HSV-1 and HSV-2 share strong amino acid sequence homology: 65% for gC, 73% for gE and 82% for gD explaining, at least in part the cross-protection against HSV-1. Second, HSV-1 appears to be

**Table 1.** HSV-2 trivalent protein vaccine efficacy against HSV-1 genital challenge.

Outcome	HSV-1 NS 1x10 <sup>6</sup>		HSV-1 17syn <sup>+</sup> 5x10 <sup>5</sup>	
	Mock	Trivalent	Mock	Trivalent
Death	0/8 (0%)	0/9 (0%)	0/5 (0%)	0/5 (0%)
Animals with positive vaginal titers days 2 or 4	7/8 (88%)	6/9 (67%)	4/5 (80%)	3/5 (60%)
Animals with genital disease*	4/8 (50%)	2/9 (22%)	3/5 (60%)	0/5 (0%)
Total days with genital lesions*	15/424 (3.5%) <sup>‡</sup>	2/477 (0.4%) <sup>‡</sup>	17/135 (12.6%) <sup>‡</sup>	0/135 (0%) <sup>‡</sup>
Animals with vaginal shedding of HSV-1 DNA (excluding days 2 & 4) <sup>#</sup>	3/8 (38%)	0/9 (0%)	2/5 (40%)	0/5 (0%)
Total days of recurrent shedding of HSV-1 DNA <sup>#</sup>	4/160 (2.5%) <sup>□</sup>	0/180 (0%) <sup>□</sup>	4/65 (6%)	0/65 (0%)

\*Genital disease was monitored for 53 days in the NS group and 27 days in the 17syn<sup>+</sup> group. <sup>#</sup>Shedding of HSV-1 DNA was monitored for 20 days between days 28–49 in the NS group and 13 days between days 15–27 in the 17syn<sup>+</sup> animals; <sup>‡</sup> $P = 0.0008$ ; <sup>□</sup> $P = 0.0481$ ; <sup>‡</sup> $P < 0.0001$ ;  $P$  values were calculated by two-tailed Fisher's Exact test.



**Figure 2.** Trivalent gC2/gD2/gE2 subunit protein immunization protects guinea pigs against HSV-1 17syn<sup>+</sup> intravaginal infection. Female Harley strain guinea pigs were immunized intramuscularly as in Figure 1. Two weeks after the final immunization, the guinea pigs were challenged with  $5 \times 10^5$  PFU of HSV-1 strain 17syn<sup>+</sup>. (A) Vaginal swabs were obtained for virus titers two days and four days post-infection. The dashed line indicates the limit of detection of 3.3 PFU/mL. (B) Viral DNA was isolated from the vaginal swabs and the DNA copy number determined by qPCR. The dashed line represents the assay limit of detection at 200 copies/mL. (C) Guinea pigs were observed for genital disease for 27 days and the cumulative lesion scores plotted. (D) Vaginal swabs were obtained for vaginal shedding of HSV-1 DNA for 13 days from days 15–27 post-infection.

less virulent than HSV-2 as a genital pathogen in guinea pigs. Mock-immunized guinea pigs infected with  $1 \times 10^6$  HSV-1 NS or  $5 \times 10^5$  HSV-1 17syn<sup>+</sup> developed genital lesions in only 50% or 60% of animals, respectively. The lesions were not severe and no animal died. In contrast, we previously reported that almost all mock-immunized guinea pigs infected with  $5 \times 10^5$  PFU of HSV-2 MS developed severe genital disease and more than half succumbed by 10 days post-infection.<sup>15,16</sup> Differences between HSV-1 and HSV-2 were also noted for vaginal shedding of HSV DNA during the recurrent phase of infection. HSV-1 DNA was detected on 4/160 (2.5%) days in mock-immunized animals between days 28–49 compared to our prior reports that detected HSV-2 DNA shedding in mock-immunized guinea pigs on 10% of days in one study and 36% in another.<sup>15,29</sup>

The natural history of HSV recurrences has been reported in humans simultaneously infected by the same HSV isolate at oral and genital sites.<sup>30</sup> HSV-1 appears better adapted to oral tissues than genital tissues in that the virus reactivates 6-fold more frequently in oral than genital sites. The opposite applies to HSV-2 in that it reactivates 330-fold more often in genital than oral sites. The fact that HSV-1 adapts better to oral than genital tissues may explain the potent protection provided by an HSV-2 genital vaccine against HSV-1. Recently, we reported that gC2/gD2/gE2 nucleoside-modified mRNA

encapsulated in lipid nanoparticles provided better protection against genital HSV-2 infection in mice and guinea pigs than the same antigens expressed as baculovirus proteins administered with CpG/alum.<sup>16</sup> Future studies will determine whether the trivalent nucleoside-modified mRNA vaccine also outperforms the trivalent protein vaccine in protecting against HSV-1 genital infection.

## Acknowledgments

We thank Carolyn Shaw and Fushan Wang for technical assistance and Gary Cohen for providing the gC2, gD2, gE2 subunit protein antigens. This work was supported by National Institute of Allergy and Infectious Disease Grants RO1 AI104854 and RO1 AI139618 (both to HF) and National Institute of Neurological Disorders and Stroke training grant T32 NS007180 (to KE).

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

## Funding

This work was supported by the National Institutes of Health (US) [RO1 AI139618]; National Institutes of Health (US) [T32 NS007180]; National Institutes of Health (US) [RO1 AI104854].

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