DNA Immunization against Experimental Genital Herpes Simplex Virus Infection

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A nucleic acid vaccine, expressing the gene encoding herpes simplex virus (HSV) type 2 glycoprotein D (gD2) under control of the cytomegalovirus immediate-early gene promoter, was used to immunize guinea pigs against genital HSV-2 infection. The vaccine elicited humoral immune responses comparable to those seen after HSV-2 infection. Immunized animals exhibited protection from primary genital HSV-2 disease with little or no development of vesicular skin lesions and significantly reduced HSV-2 replication in the genital tract. After recovery from primary infection, immunized guinea pigs experienced significantly fewer recurrences and had significantly less HSV-2 genomic DNA detected in the sacral dorsal root ganglia compared with control animals. Thus, immunization reduced the burden of latent infection resulting from intravaginal HSV-2 challenge, and a nucleic acid vaccine expressing the HSV-2 gD2 antigen protected guinea pigs against genital herpes, limiting primary infection and reducing the magnitude of latent infection and the frequency of recurrent disease.

Herpes simplex virus (HSV) infections are among the most common communicable diseases of humans [1, 2], and the need for a vaccine is well recognized. Most research is currently focused on the development of viral subunit vaccines (reviewed in [3]), but other approaches are being evaluated, including the use of live attenuated [4] and replication-incompetent viruses [5, 6]. The recent development of nucleic acid–based vaccines has provided a novel approach to controlling infectious diseases [7].

The observation that intramuscular injection of plasmid DNA expression vectors resulted in the in vivo expression of the encoded protein in mice [8] was followed by studies exploring the mechanism and timing of gene expression [9]. In addition, studies have shown protein expression after DNA delivery by a variety of other routes, including intradermal and mucosal [10–13]. As vaccines, nucleic acid injections were shown to elicit both cell-mediated and humoral immune responses to the expressed protein [9, 12, 14]. Protection against viral infection was first demonstrated in an experimental influenza virus infection model in mice [7]. Subsequent studies showed that immunization with other influenza genes could protect not only mice but also chickens and ferrets against experimental influenza virus challenge, thus demonstrating that nucleic acid vaccines were effective in multiple species [11, 12, 14, 15]. Animal studies have also demonstrated the protective efficacy of DNA against other viral and nonviral pathogens, including HSV and rabies virus in mice, bovine herpesvirus in cattle, and Leishmania and Plasmodium species in mice [16–20].

Here we describe evaluation of a DNA vaccine for protection against HSV-2 challenge in the guinea pig model of genital herpes. Genital HSV infection in the guinea pig is very similar to genital herpes in humans [21–23]. After inoculation via a natural route (either intravaginal or intraurethral), virus replicates in the genital tract and spreads via sensory neurons to the sacral dorsal root ganglia, where latency is established. Animals typically develop a vesiculoulcerative primary genital skin disease that is self-limiting but is followed by the development of episodic spontaneous recurrent genital lesions [22]. Because it shares many similarities with the human disease, this model has been extensively used for the preclinical evaluation of both vaccines and antiviral agents [24–29]. Previous studies have shown that subunit glycoprotein vaccines formulated with potent adjuvants can induce high-titer antibody responses and protect animals against both primary and recurrent disease [25–27]. These vaccines reduce viral replication in the genital tract but do not prevent infection [26].

Using a plasmid encoding the HSV-2 glycoprotein D (gD2) gene under the control of the human cytomegalovirus (CMV) immediate-early gene promoter, we examined the effects of intramuscular injection on induction of humoral immunity and protection against primary, recurrent, and latent infection.

Methods and Materials

Virus and cells. The HSV-2 strain MS (American Type Culture Collection, Rockville, MD) used in the animal study was prepared by culture in low-passage RK cells. Stock virus was...
maintained frozen at -70°C. Primary RK cells were prepared from New Zealand White rabbits (Hazleton Research Products, Denver, PA) and maintained in Eagle’s basal medium supplemented with 10% fetal bovine serum (FBS). BHK cells were obtained from American Type Culture Collection.

Construction of the HSV-2 gD expression vector VR-2110. The full-length gD2 gene (figure 1) was cloned from purified genomic HSV-2 strain MS DNA by polymerase chain reaction (PCR) amplification using the following primers: sense, 5'-GTTCGGTTCA-TAACGTCAAGTGCGAACCACCTAGTCGCGG-3'; antisense: 5'-CCTAGTTCTCCCTCTAGACTCCCCCCATGCGG-3'.

PCR amplification was done in a thermocycler (GeneAmp PCR System 9600; Perkin-Elmer Cetus, Norwalk, CT) for one cycle of 94°C for 3 min, 58°C for 1.5 min, and 72°C for 1.5 min, followed by 30 cycles of 94°C for 1 min, 58°C for 1.5 min, and 72°C for 1.5 min. The final concentrations of all PCR components in a 100-μL volume were as follows: 3 ng of purified HSV-2 DNA, 200 μM each dNTP, 100 pmol of each primer, and 1 U of Taq polymerase (Stratagene, La Jolla, CA) in Taq polymerase buffer (10 mM TRIS [pH 8.3], 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin). The PCR product was 1356 nt, including 64 nt of the 5' untranslated region, the entire HSV-2 gD coding sequence including the signal sequence, and 109 nt of the 3' untranslated region. The PCR product was initially cloned into a pCR II TA cloning vector (Invitrogen, San Diego). A PstI/partial BamHI fragment was subsequently cloned into vector VCL-1010, which contains the human CMV immediate-early gene promoter [30-32] and intron A [33]. In addition, VCL-1010 possesses the bovine growth hormone polyadenylation processing signal [34] and the gene encoding kanamycin resistance. The resulting HSV-2 gD2 eukaryotic expression plasmid VR-2110 is shown in figure 2 (top). A control plasmid, kCMVint-BL, containing the CMV promoter and intron with an SV40 polyadenylation signal was also prepared (figure 2, bottom).

In vitro evaluation. Plasmid VR-2110 was transfected into BHK cells using cationic lipid-based delivery [36]. Briefly, 5 μg of plasmid DNA was mixed with the cationic lipid DMRIE (1,2-dimyristoyl-3-dimethyl ammonium bromide) [37] and the neutral lipid DOPE (dioleoyl phosphatidylethanolamine) [38] at a lipid/DNA mass ratio of 2:1 in 2 mL of OptiMEM (Life Technologies Gibco BRL, Gaithersburg, MD). The mixture was added to 5 × 10^5 BHK cells in 60-mm dishes and incubated at 37°C in a 5% CO2 incubator. After 4 h, an additional 2 mL of OptiMEM (Life Technologies) was added with 20% FBS to a final FBS concentration of 10%, and the cells were incubated at 37°C for 48 h. Cytoplastic extracts were prepared from transfected cells by the method of Chiang et al. [39]. Culture media (20 μL) or cytoplasmic extract (10 μL) was run on a 12% polyacrylamide Laemmli gel at 100 V for 1 h. The gel was transferred to a polyvinylidene fluoride transfer membrane (Immobulin; Millipore, Bedford, MA) in 20 mM TRIS (pH 8.3), 150 mM glycine, and 20% methanol at 150 V for 2 h using an electrotransfer apparatus (BioRad, Hercules, CA). After transfer, the proteins were fixed to the membrane in 100% methanol. The membrane was equilibrated in

Figure 1. Full-length gene of HSV-2 glycoprotein D (gD2). Arrows, position of polymerase chain reaction primers. Bold bars, coding regions for gD and morphologic transforming region (mtr-2) genes. TR, terminal repeat; U, unique; IR, internal repeat; S, short; L, long.

Figure 2. A, Map of plasmid VR-2110, containing eukaryotic cytomegalovirus immediate-early gene enhancer and promoter (CMV promoter) plus intron A (CMV intron) driving expression of gene encoding HSV-2 glycoprotein D (HSV gD2). Transcription is terminated by bovine growth hormone polyadenylation and termination signal [BGHp(A)]. Kanamycin resistance gene is also indicated (kan). B, Map of negative control plasmid, kCMVintBL, containing same CMV enhancer/promoter plus intron A, but no coding sequence. It possesses SV40 small t intron and polyadenylation signal [SV40p(A)].
lected 4 weeks after intravaginal HSV-2 inoculation (natural immunization), as well as sera from 2 unimmunized animals. Collected purified glycoproteins were used as the solid phase, and peroxidase-conjugated rabbit anti-guinea pig immunoglobulin (Accurate Chemical, Westbury, NY) was used in the assay. Serum was also analyzed from 2 animals that had received two instillations of HSV-2 strain 333. The neutralizing titer was defined as the reciprocal of the highest dilution of serum to produce an observable neutralization effect.

In vivo evaluation. Forty-eight female Hartley guinea pigs (Charles River Breeding Laboratories, Wilmington, MA) were randomized to 8 groups (n = 6 each). On days 0, 21, and 42, animals were immunized by bilateral intramuscular injection into the rear legs with sterile saline solution containing either control plasmid (kCMVint-BL) or the gD2 plasmid (VR-2110). The amount of DNA and volume per immunization were as follows: group 1 and 2, 250 µg of control plasmid in 250 µL or 1000 µL, respectively; groups 3 and 4, 50 µg of gD2 plasmid in 100 µL or 1000 µL, respectively; groups 5 and 6, 100 µg of gD2 plasmid in 100 µL or 1000 µL, respectively; groups 7 and 8, 250 µg of gD2 plasmid in 250 µL or 1000 µL, respectively.

Serum was collected from all animals on days 0, 21, 42, and 63. Samples were stored frozen (−20°C) until used in ELISA and neutralization assays.

On day 64, animals were inoculated by intravaginal instillation of HSV-2 strain MS. Briefly, the vaginal closure membrane was ruptured with a moistened calcium alginate–tipped swab (Calgiswab no. 3; Spectrum Laboratories, Los Angeles), and 0.1 mL of a suspension containing 5 × 10⁷ pfu of virus was instilled into the vaginal vault using a plastic catheter (Abbocath; Abbott, Abbott Park, IL). To ensure maximal infection, the instillation procedure was done twice. To measure the course of viral replication in the genital tract, vaginal swab samples were collected on days 1, 2, 3, 5, and 7 after inoculation and stored frozen (−70°C) until assayed for virus by titration on primary RK cells. Guinea pigs were evaluated daily, and the severity of primary genital skin disease was quantified using a lesion score scale ranging from 0 for no disease to 4 representing severe vesiculoulcerative disease of the perineum [21]. After recovery from primary disease, animals were observed daily from days 15 to 42 after inoculation for evidence of spontaneous recurrent herpetic lesions [22].

Serology. Antibody to HSV-2 was determined by ELISA as described [40]. Briefly, lectin-purified glycoproteins prepared from HSV-2–infected or uninfected HEP-2 cell monolayers were used as the solid phase, and peroxidase-conjugated rabbit anti–guinea pig immunoglobulin (Accurate Chemical, Westbury, NY) was used for detection of guinea pig antibody. Neutralizing antibody to HSV-2 was determined as described [23] with the exception that the virus used in the assay was HSV-2 strain 333. The neutralizing titer was calculated as the reciprocal of the highest dilution of serum to produce a 50% reduction in the number of plaques. For purposes of comparison, sera were also analyzed from 2 animals that had received two doses of an HSV-2 mixed glycoprotein vaccine combined with complete Freund’s adjuvant (sera collected 2 weeks after the second immunization), as well as sera from 2 unimmunized animals collected 4 weeks after intravaginal HSV-2 inoculation (natural infection). These samples were collected as part of a subunit vaccine evaluation reported by Bernstein et al. [40].

Detection of DNA in guinea pig tissues. Between days 48 and 51 after inoculation, animals were sacrificed and sacral dorsal root ganglia collected for PCR analysis of latent viral DNA as described [41]. In addition, a portion of the left rear quadriceps muscle at the site of immunization was removed from each animal for PCR detection of plasmid DNA. All tissue samples were frozen immediately after collection and stored frozen (−70°C) until DNA extraction. Total DNA was isolated from tissue samples and incubated overnight at 65°C with 0.5 mL of 50 mM TRIS-HCl (pH 8.0), 100 mM EDTA, 100 mM NaCl, and 1% (wt/vol) SDS solution containing 0.25 mg of proteinase K (Boehringer-Mannheim, Indianapolis). After extraction with equal volumes of phenol and chloroform, samples were precipitated at room temperature with 2 vol of ethanol, followed by centrifugation at 12,000 g for 10 min. The pellets were rinsed with 70% ethanol and dried in a laminar flow hood at ambient temperature. Pellets were resuspended in 100 µL of water containing 0.1 mg/mL RNase A (Sigma, St. Louis) and incubated at 37°C for 30 min, and the DNA was precipitated and dried as described above. DNA concentrations were determined spectrophotometrically. PCR amplification conditions were the same as described above for construction of the gD2 plasmid, except that 1 µg of total DNA from isolated tissue was used for each reaction.

For PCR analysis of DNA from dorsal root ganglia, the primers used were specific for the morphologic transforming region of HSV-2 (mtr-2; figure 1): mtr-2 sense primer: 5’-TCACTCGCC-AGTCGAAGCCT-3’; mtr-2 antisense primer: 5’-CGACGC-GGTAGCTCTTCAAC-3’. PCR reaction products were separated electrophoretically on 2% agarose with TAE running buffer (40 mM TRIS acetate, pH 8.5, 2 mM EDTA). The products were transferred to hybridization membranes (Nytran Plus; Schleicher & Schuell, Keene, NH) by Southern transfer and hybridized to a 32P-labeled probe amplified from the HSV-2 mtr. Quantitative analysis was done with a Phosphorimager SI (Molecular Dynamics, Sunnyvale, CA). The quantity of viral DNA in the ganglia of each animal was determined from a linear regression analysis of a standard curve of viral DNA. Animals in which the amount of viral DNA fell below the limit of the linear portion of the curve were assigned a value of 1 fg of viral DNA.

DNA extracted from the quadriceps muscle was analyzed for the presence of plasmid DNA by use of primers specific for the kanamycin resistance gene, which has no known sequence homology for HSV-2 or guinea pig genomic DNA: sense, 5’-GCGAAC-ATCCGTGATATCGGT-3’; antisense, 5’-CGTACTCCTGAT-GTGCAATGG-3’. PCR reaction products from muscle DNA were separated by agarose gel electrophoresis and identified by ethidium bromide staining.

Statistics. For comparisons between 2 groups, data were analyzed by Student’s t test or Fisher’s exact test as appropriate. Comparisons of multiple groups were made by one-way analysis of variance with Bonferroni correction. All comparisons are two-tailed.

Results

In vitro expression of HSV gD2 plasmid VR-2110. After transient transfection of plasmid VR-2110 into BHK cells,
HSV-2 gD was detected in both culture media and cytoplasmic extract by Western blot analysis using the HSV gD2–specific polyclonal antibody R7 (figure 3). These results indicated that transcription and translation of the plasmid DNA by BHK cells produced an immunogenic protein of the correct size. Culture media and cytoplasmic extract from cells transfected with plasmid VCL-1010, which does not contain the gD gene, showed no immunoreactive product.

Serology. After one immunization, only 3 of 12 animals receiving the 50-μg dose of DNA developed anti-HSV antibody detected by ELISA, compared with 21 of 24 animals receiving higher DNA doses. After the second DNA immunization, 35 of 36 immunized animals developed antibody, and after the third immunization, all animals immunized with the gD2 plasmid developed anti-HSV antibody (figure 4). None of the animals receiving the control plasmid, kCMVintBL, developed antibodies to HSV. Responses in all groups were higher after the three-dose regimen than those seen in animals after natural infection but lower than those produced by a mixed HSV-2 glycoprotein vaccine containing complete Freund’s adjuvant. The mean ELISA antibody titer for group 3, which received the lowest dose of gD2 plasmid in the smallest volume (50 μg in 100 μL), was significantly lower than titers in other immunized groups (by analysis of variance with the Bonferroni correction for multiple groups). The DNA dose and injection volume did not significantly affect ELISA responses except for those of animals in group 3.

Neutralizing antibody was also detected in animals immunized with the gD2 plasmid (figure 4). Mean titers after the third immunization were comparable with those seen after natural infection but were again lower than those elicited by immunization with viral glycoproteins and adjuvant. DNA dose and injection volume did not affect neutralizing antibody titers.

Viral replication in the genital tract. Animals that did not develop symptomatic primary or recurrent genital skin disease were defined as infected if virus was detected in vaginal secretions on at least 2 of the first 3 days after inoculation. With this definition, 29 of 36 immunized animals and 10 of 12 control guinea pigs were evaluated. For all three concentrations of DNA tested, the volume used for delivery had no effect on viral replication in the genital tract (data not shown). Therefore, results for both volumes at each concentration were combined for analysis. DNA immunization did not prevent viral replication in the vagina at any of the concentrations tested (table 1). However, peak virus titers, seen on day 1 after inoculation, were significantly reduced in all immunized groups compared with controls (P < .001; table 1). In addition, the magnitude of viral replication as measured by the area under the virus titer–day curve was also significantly reduced in all groups (P < .01; table 1). There were no significant differences in peak virus titer or magnitude of replication between the three DNA vaccine concentrations used.

Primary and recurrent genital disease. The volume used for DNA immunization had no impact on primary genital skin disease (defined as first episode genital skin disease by day 14 after inoculation) or, subsequently, on recurrent disease (data not shown). Therefore, results for the two volumes at each DNA concentration used were combined for analysis. Immunization significantly reduced the incidence of primary genital skin disease at each concentration tested (table 1). Overall, symptomatic primary disease developed in 9 of 10 infected control animals but in only 6 of 29 infected immunized animals (P < .001). Two control animals died as a result of primary disease, while there was no mortality among immunized animals. Control animals also experienced more severe primary disease (as measured by the area under the lesion score–day curve) than symptomatic immunized animals (P < .002). Indeed, while all control animals developed vesicular lesions, such lesions were seen in only 3 of the 6 symptomatic immunized animals, the other 3 developing only erythema. Furthermore, in only 1 immunized animal did the vesicular lesions persist for >1 day, compared with a mean duration of 4.7 ± 0.6 days for control animals.

Recurrent disease developed in 7 of the 8 surviving control animals compared with 9 of the 29 immunized guinea pigs that became infected, including 3 that had experienced symptomatic primary infection. Thus, the incidence of recurrent disease was significantly reduced in all immunized animals (9/29 vs. 7/8; P = .007) and at each DNA concentration tested (table 1). Among animals that did experience recurrences, the frequency of recurrent disease was significantly reduced in all groups immunized with the gD2 plasmid (table 1).

PCR analysis. At the conclusion of the study, HSV DNA in the sacral dorsal root ganglia of surviving, infected animals was detected and quantified by PCR analysis. Viral DNA was detectable in all of the animals; however, for 7 of the immunized guinea pigs, the amount of DNA was at the limit of detection. There was significantly less viral DNA present in the ganglia of the 29 immunized animals (252 ± 202.0 fg/μg of genomic DNA/animal, mean ± SE) compared with the 8 controls (1821 ± 639 fg; P = .0037). This reduction in latent viral DNA paralleled the reduction in recurrent disease seen in immunized animals. Although there was considerable animal-to-animal variation in the amount of viral DNA detected,
similar results have been seen in previous studies (unpublished observation).

Control (kCMVintBL) and gD2 (VR-2110) plasmid DNA was detected by PCR analysis in the DNA isolated from the quadriceps muscles of all injected animals (data not shown).

Discussion

In this study, we constructed a plasmid (VR-2110) containing the HSV-2 gD gene under the control of the CMV immediate-early gene promoter. The gD2 gene was chosen because glycoprotein D is one of the major immunogenic proteins of HSV and because gD2 subunit vaccines have proven both immunogenic and protective in the guinea pig model (reviewed in [3]). Initial in vitro studies showed that cells transfected with VR-2110 produced gD2. Guinea pigs immunized by intramuscular injection developed HSV-2–specific ELISA and neutralizing antibody responses. ELISA titers in VR-2110–immunized animals were higher than those resulting from natural infection, and neutralizing antibody titers were comparable to those in natural infection, while both ELISA and neutralizing titers were lower than those seen in animals immunized with HSV-2 glycoproteins combined with Freund’s adjuvant.

We examined the effect of injection volume on the efficacy of the DNA vaccine. Volume of injection affected the ELISA titer responses produced by the lowest concentration of gD2 plasmid DNA (50 μg), with the smallest injection volume (100 μL) eliciting a significantly lower titer than the larger volume (1000 μL). However, this was the only effect observed. For the 50-μg dose, injection volume did not affect neutralizing antibody titers, nor did the volume affect neutralizing or ELISA antibody titers with the 100- and 250-μg DNA doses. The injection volume also did not affect viral replication in the genital tract or the severity of

Figure 4. Antibody response to DNA immunization as quantitated by ELISA or neutralization titers (mean ± SD). CFA, complete Freund’s adjuvant. A, Significant difference vs. groups receiving >100 μg (P < .05); B, significant difference vs. group receiving 50 μg/100 μL (P < .001); C, significant difference vs. group receiving 50 μg/1000 μL (P < .05).
primary genital skin disease at any DNA concentration evaluated. We concluded that the injection volume of the DNA vaccine did not affect efficacy in the guinea pig model of genital herpes. As there was no significant volume effect, the 2 groups receiving different volumes but the same dosage of DNA were analyzed as a single group.

DNA immunization significantly reduced viral replication in the genital tract, affecting both the peak and magnitude of replication. The incidence of symptomatic primary genital herpes was also significantly reduced, with most immunized animals exhibiting no evidence of genital infection. For those immunized guinea pigs that developed skin lesions, the skin disease was significantly less severe than the disease observed in control animals. Likewise, the incidence of recurrent genital herpes was significantly reduced in immunized animals at all concentrations of DNA tested, and those animals that did experience recurrences had significantly fewer lesion days than did controls. Furthermore, DNA immunization significantly reduced the magnitude of latent infection as measured by the concentration of HSV-2 DNA in the sacral dorsal root ganglia of latent infected animals. Our results show that even the lowest dose of the gD2 plasmid (50 μg) provided excellent protection against symptomatic disease. However, infection was not prevented at any dosage, as evidenced by the detection of virus in vaginal swab samples from asymptomatic animals.

Similar to our findings, data from Manickan et al. [16] showed that intramuscular injection of mice with plasmid DNA encoding HSV-1 glycoprotein B protected 80% of the animals against zosteriform (cutaneous) HSV-1 infection and that lesion development was delayed in the remaining mice. Immunized animals developed HSV-specific antibody responses, including low levels of neutralizing antibody. The investigators presented equivocal data suggesting a cytotoxic T cell (CD8 cell) response but compelling data that immunization induced a protective CD4 T lymphocyte response. Our study establishes the immunogenicity and efficacy of an HSV DNA vaccine in a second species (guinea pig) and demonstrates that a nucleic acid vaccine can protect against intravaginal challenge.

The protection afforded by DNA immunization in our study, at all three DNA doses tested, was comparable with that seen previously in the guinea pig model of genital herpes using glycoprotein vaccines and potent adjuvants [25–27]. Immunization of animals with subunit vaccines before intravaginal HSV-2 infection has been reported to affect the establishment of latency. Using in situ hybridization, Burke et al. [29] reported that the HSV-2 latency-associated transcript (LAT RNA) was detected in the lumbosacral dorsal root ganglia of 4 of 19 immunized animals compared with 5 of 5 untreated controls. Recent reports have shown that PCR techniques are more sensitive than in situ hybridization for detecting latent virus [42] and have the advantage of being quantitative [43]. In this study, using quantitative PCR methods, we were able to demonstrate that prophylactic immunization with a DNA vaccine reduced the amount of latent viral DNA in the ganglia, a finding that paralleled the observed reduction in recurrent disease.

In conclusion, we have shown that intramuscular immunization with plasmid DNA containing the gD2 gene by intramuscular injection was both immunogenic and protective in the guinea pig model of genital HSV-2 infection. This is the first demonstration of the prophylactic effects of DNA immunization in guinea pigs and, we believe, the first report establishing that prophylactic administration of a DNA vaccine can interfere with the establishment of latent HSV infection. Further studies to optimize this vaccine strategy are warranted. Such studies could include systematic modifications of the DNA vector with the aim of increasing expression at the transcriptional and post-transcriptional levels, possibly by the use of tissue-specific enhancers, and the addition of sequences to assist in message stability. Additional characterization of the host immune responses and durability of immunity induced by DNA vaccines is also warranted.

Table 1. Effect of DNA immunization on experimental genital HSV-2 infection.

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<tr>
<th>Plasmid (μg of DNA)</th>
<th>Vaginal viral replication</th>
<th>Primary disease</th>
<th>Recurrent disease</th>
</tr>
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<tr>
<td>Control (250)</td>
<td>10/12</td>
<td>26.8 ± 0.9</td>
<td>7/8</td>
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<tr>
<td>gD2 (50)</td>
<td>9/12</td>
<td>14.4 ± 2.4</td>
<td>3/11</td>
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<tr>
<td>gD2 (100)</td>
<td>11/12</td>
<td>16.7 ± 2.3</td>
<td>2/11</td>
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<tr>
<td>gD2 (250)</td>
<td>9/12</td>
<td>13.6 ± 2.4</td>
<td>2/9</td>
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NOTE. *gD2, glycoprotein D of HSV-2.

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<th></th>
<th>Vaginal viral replication</th>
<th>Primary disease</th>
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<tr>
<td></td>
<td>Frequency**</td>
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<td>Mean ± SE; log10 pfu; calculated using only animals that experienced viral replication.</td>
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<tr>
<td>Mean ± SE; as measured by area under virus titer–day curve. Calculated using only animals that experienced viral replication.</td>
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<tr>
<td>No. of animals with primary genital skin disease/no. infected.</td>
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<tr>
<td>No. of animals with recurrent genital skin disease/no. infected animals available.</td>
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** Mean ± SE; recurrent lesion days between days 15–42 after inoculation. Calculated using only animals that experienced recurrences. P < ° .001, ° .01, °° .002, °° .05, and °° .02 vs. controls.
Acknowledgments

We thank Gary H. Cohen and Roselyn J. Eisenberg, University of Pennsylvania, for their gift of the R7 polyclonal antibody to HSV-2 gD2; Peter Hobart, Vical, for thoughtful manuscript review; and Frances Childs, Alisa Reese, James Ireland, Terrie Latimer, and Greg Milligan for excellent technical assistance.

References