Comparison of Different Forms of Herpes Simplex Replication-Defective Mutant Viruses as Vaccines in a Mouse Model of HSV-2 Genital Infection

Xavier J. Da Costa,*1 Lynda A. Morrison,† and David M. Knipe*2

*Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, Massachusetts 02115; and †Department of Molecular Microbiology and Immunology, Saint Louis University School of Medicine, 1402 South Grand Boulevard, St. Louis, Missouri 63104

Received March 12, 2001; returned to author for revision June 6, 2001; accepted July 11, 2001

Some subunit vaccines composed of herpes simplex virus (HSV) glycoproteins have been shown to protect guinea pigs against primary and recurrent genital infection by HSV-2. However, these vaccines were ineffective or only marginally effective in clinical trials. To attempt to define an animal model that would better discriminate the protective capacity of different vaccine formulations, we have examined the requirements for vaccine-induced protection against HSV-2 infection and disease in a mouse genital model. Unlike the guinea pig model where inactivated viral vaccines can protect nearly as well as live viral vaccines, inactivated viral vaccine afforded little protection in this mouse model. Using replication-defective mutant viruses as a form of live viral vaccine, we found that the extent of protection conferred by live vaccine was proportional to the amount of replication-defective mutant virus inoculated, over doses from 104 to 106 PFU. Furthermore, the mouse genital model showed quantitative differences in the degree of protection induced by various viral vaccine constructs. An HSV-2 replication-defective mutant virus protected better than an HSV-1 replication-defective mutant that expressed HSV-2 glycoprotein D, which in turn protected better than an HSV-2 replication-defective mutant virus. We conclude that this mouse genital model can rank different vaccine constructs for their capacity to induce protective immunity. Thus, genital infection of the mouse with HSV-2 may provide a stringent animal model that can predict the relative capacity of viral vaccines to stimulate protective immunity against HSV-2.

INTRODUCTION

Herpes simplex virus 2 (HSV-2) is the principal etiologic agent of genital herpes. This disease causes a significant amount of morbidity in infected individuals and in neonates who contract severe, disseminated infections from their actively infected mothers. Fetuses at risk for contracting HSV are usually delivered by Cesar-ean section as a means of prevention, adding significantly to health care costs. A recent study reported a 30% increase in the incidence of HSV-2-mediated genital disease since the late 1970s; thus, at this time approximately one in five Americans over the age of 12 is seropositive for HSV-2 (Fleming et al., 1997). Individuals may be unaware of their infection, often resulting in silent transmission to susceptible partners and contributing to the high rate of infection.

Numerous approaches, including the use of glycoprotein subunits, inactivated virus, attenuated mutant HSV strains, DNA, and recombinant viral vectors expressing HSV antigens, have been utilized for immunization against HSV infection and disease in animal model systems (reviewed in Bernstein and Stanberry, 1999; Krause and Straus, 1999; Stanberry et al., 2000). In particular, the guinea pig genital model of HSV-2 infection has been regarded as a useful model for human infection because primary and recurrent infections have been defined (Stanberry et al., 1982, 1985). Glycoprotein subunit vaccines have proven effective for inducing protective immunity in guinea pigs against primary and recurrent genital infection by HSV-2 (Byars et al., 1994; Stanberry et al., 1987, 1989a, 1989b). Furthermore, in some experiments inactivated viral vaccine induced protection in guinea pigs that was nearly equivalent to that produced by live viral vaccine (Da Costa et al., 1997; McLean et al., 1994). However, the glycoprotein subunit vaccines were ineffective at inducing protective immunity in clinical trials involving prophylactic (Corey et al., 1999; Mertz et al., 1990) or therapeutic immunization (Straus et al., 1997). Furthermore, an attenuated HSV strain that induced protective immunity in mice against intracranial (ic) challenge (Meignier et al., 1988) was not immunogenic in human trials (Cadoz et al., 1992). Because of this general experience, concerns have been raised about the lack of a discrerning animal model for testing herpes vaccine candidates (Mascola, 1999).

HSV-1 and HSV-2 replication-defective mutant viruses have induced protective immunity in animal studies against lethal intraperitoneal challenge with HSV-1...
(Nguyen et al., 1992), ocular challenge with HSV-1 (Morrison and Knipe, 1994), inoculation of HSV-1 into murine ear pinnae (Farrell et al., 1994), and HSV-2 genital challenge (Boursnell et al., 1997; Da Costa et al., 1997, 1999; McLean et al., 1994, 1996; Morrison et al., 1998). We have used a mouse genital infection model (McDermott et al., 1984) as modified by Parr et al. (1994) to study the effect of immunization on HSV-2 challenge virus infection. In this study we asked whether this mouse system could distinguish live from inactivated replication-defective mutant virus, whether the protection was dose-dependent, and whether the system could distinguish different mutant viral strains in terms of protective immunity. We observed that this mouse model of immunization and subsequent genital infection does permit us to perceive differences in primary infection, disease severity, and lethality of challenge virus infection after immunization with different virus vaccines.

RESULTS
Characterization of the mouse genital model system

In the herpes simplex vaccine field there is a clear need for an animal model that can be used to test the relative efficacy of different vaccine constructs for inducing protective immunity. As described above, glycoprotein subunit vaccines that protected against primary and recurrent genital disease in guinea pigs were not efficacious in clinical trials. Thus, an animal model in which protection is not induced by glycoprotein subunits or inactivated virus might better predict the human situation.

We have used a modified version of the mouse genital infection model developed by Parr et al. (1994) to demonstrate the ability of HSV-2 replication-defective mutant strains to induce protective immunity against genital challenge with virulent HSV-2 (Da Costa et al., 1999; Morrison et al., 1998). The latter two studies showed that subcutaneous and/or intranasal inoculation of the HSV-2 5BlacZ mutant virus could induce protective immunity against genital infection. 5BlacZ is an HSV-2 mutant virus strain in which the UL29 coding sequences are partially replaced with lacZ coding sequences, rendering the virus replication-defective in normal cells (Da Costa et al., 1997). To determine whether inactivated virus could also confer protection in this mouse genital model, we compared the capacities of live and UV-inactivated 5BlacZ mutant virus to immunize against genital challenge. Female BALB/c mice were immunized subcutaneously (sc) with \(10^6\) PFU of 5BlacZ virus, an equivalent amount of UV-inactivated 5BlacZ or control preparation. Mice were challenged i.vag. with HSV-2 G. (A) Virus shedding from the genital tract. Values represent the geometric mean ± SEM for each group. (B) Genital disease based on a 5-point scale. Values represent the arithmetic mean ± SEM for each group. (C) Survival of immunized animals.

![FIG. 1. Comparison of immunization with live versus UV-inactivated vaccine virus. Groups of six BALB/c mice were immunized sc with \(10^6\) PFU of live 5BlacZ or an equivalent amount of UV-inactivated 5BlacZ or control preparation. Mice were challenged i.vag. with HSV-2 G. (A) Virus shedding from the genital tract. Values represent the geometric mean ± SEM for each group. (B) Genital disease based on a 5-point scale. Values represent the arithmetic mean ± SEM for each group. (C) Survival of immunized animals.](image-url)

FIG. 1. Comparison of immunization with live versus UV-inactivated vaccine virus. Groups of six BALB/c mice were immunized sc with \(10^6\) PFU of live 5BlacZ or an equivalent amount of UV-inactivated 5BlacZ or control preparation. Mice were challenged i.vag. with HSV-2 G. (A) Virus shedding from the genital tract. Values represent the geometric mean ± SEM for each group. (B) Genital disease based on a 5-point scale. Values represent the arithmetic mean ± SEM for each group. (C) Survival of immunized animals.

to nearly undetectable levels (\(P = 0.0053\)). In contrast, the same amount of UV-inactivated 5BlacZ reduced viral shedding by only about 10-fold over time (Fig. 1A; \(P = 0.3015\)). Similarly, UV-inactivated vaccine virus provided only partial protection against genital disease (Fig. 1B; \(P > 0.05\)) and lethality (Fig. 1C; \(P > 0.05\)) while live vaccine virus completely protected the animals (\(P = 0.027\) to 0.001 on days 5 through 10 for disease; \(P = 0.0011\) for death). Thus, by several parameters, inactivated vaccine provided very limited protective immunity in this mouse genital model while live vaccine provided complete protection.

To examine the quantitative nature of the system, we
determined the magnitude of the protective immunity in response to different doses of 5BlacZ virus. We immunized mice sc with 10^4, 10^5, or 10^6 PFU of 5BlacZ and then challenged the mice i.vag. with 50 LDso of HSV-2 G virus. Immunization with 10^5 PFU of 5BlacZ reduced viral shedding from the genital tract only slightly (Fig. 2A). Immunization with 10^5 PFU reduced viral shedding by 1000-fold by day 5 postchallenge (Fig. 2B), and immunization with 10^6 PFU reduced viral shedding by more than 1000-fold by day 4 and completely eliminated viral shedding by day 5 (Fig. 2C; P = 0.0172). Therefore, the level of protective immunity induced by 5BlacZ in this system, as measured by reduced challenge virus replication in the genital tract, was dependent on the dose of vaccine virus used for the immunization.

Comparison of protective immunity induced by different forms of replication-defective mutant viral vaccines

We also wished to determine whether the mouse genital infection system could be used as an assay to determine the relative efficacy of several replication-defective mutant viral strains to induce protective immunity against HSV-2 genital infection. We isolated an intertypic recombinant ICP27 mutant virus expressing both HSV-1 glycoprotein D (gD-1) and HSV-2 glycoprotein D (gD-2), dl27gD2 (Fig. 3), for comparison with the parental HSV-1 mutant dl27lacZ1 (Fig. 3) and the HSV-2 mutant 5BlacZ (Fig. 3).

To confirm that the intertypic recombinant ICP27 mutant virus dl27gD2 expressed both gD-1 and gD-2, we resolved the proteins in infected-cell lysates by SDS-PAGE, transferred them to nitrocellulose membranes, and probed the membranes with anti-gD-1 or anti-gD-2 rabbit antibody (Fig. 4). Two species of gD were detected by the gD-1 antibody (Fig. 4A). A slower migrating species was detected in cells infected with the HSV-1 mutant dl27lacZ1 while a faster migrating species was detected in cells infected with HSV-2 5BlacZ and WT HSV-2 (Fig. 4A, lanes 4 and 5). Both species were detected in cells infected with the HSV-1 recombinant dl27gD2, arguing that it expressed both gD-1 and gD-2 (Fig. 4A, lane 2). The anti-gD-2 antibody reacted more specifically with gD-2 (Fig. 4B). The HSV-1 mutant dl27lacZ1 showed no gD-specific bands with this antibody (Fig. 4B, lane 1) while dl27gD2 expressed protein that reacted with the anti-gD-2 antibody (Fig. 4B, lane 2), as did 5BlacZ and WT HSV-2. Thus, dl27gD2 expressed both gD-1 and gD-2.

We then compared HSV-1 dl27lacZ1 and HSV-2 5BlacZ to the intertypic recombinant dl27gD2 for the capacity to protect mice from a lethal genital challenge with HSV-2. All animals were immunized by sc injection in the rear flank and subjected to i.vag. challenge 4 weeks later with...
50 LD$_{50}$ of WT HSV-2, strain G. Animals were examined daily for signs of clinical disease and survival, and the amount of virus shed in the genital tract was determined by titration of vaginal swabs. As exemplified by disease scores at 7 days postchallenge, the degree of protection observed was both dose- and virus-dependent (Table 1). At the lowest immunizing dose, $10^4$ PFU, immunized animals fared no better than those that receiving control cell lysate and all animals succumbed to infection (Fig. 5A). Animals receiving $10^5$ PFU, however, showed fewer signs of disease than control animals after challenge (Table 1). At this intermediate dose, dl27gD2 protected somewhat better than dl27lacZ1 from disease and also prolonged the time of death (dl27gD2, $P = 0.0088$; dl27, $P = 0.0088$; dl27lacZ1, $P = 0.0309$) but time to death in the dl27gD2-immunized group was less than that of the 5BlacZ group (Fig. 5B; $P = 0.0078$). At the highest dose of immunizing virus, $10^6$ PFU, the difference between the levels of protection in animals receiving dl27lacZ1 or dl27gD2 and those receiving 5BlacZ was equally pronounced (Table 1; 5BlacZ, $P = 0.046$ compared with control). Moreover, the only group in which all the ani-

**TABLE 1**

<table>
<thead>
<tr>
<th>Immunizing virus</th>
<th>Dose of immunizing virus (PFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^4$</td>
</tr>
<tr>
<td>5BlacZ</td>
<td>4.1 ± 0.4$^a$</td>
</tr>
<tr>
<td>dl27gD2</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>dl27lacZ</td>
<td>4.1 ± 0.4</td>
</tr>
</tbody>
</table>

$^a$ Mean score ± SEM of disease for groups of mice ($N = 5$) assessed on a 5-point scale (see Methods and Materials).

50 LD$_{50}$ of WT HSV-2, strain G. Animals were examined daily for signs of clinical disease and survival, and the amount of virus shed in the genital tract was determined by titration of vaginal swabs. As exemplified by disease scores at 7 days postchallenge, the degree of protection observed was both dose- and virus-dependent (Table 1). At the lowest immunizing dose, $10^4$ PFU, immunized animals fared no better than those that receiving control cell lysate and all animals succumbed to infection (Fig. 5A). Animals receiving $10^5$ PFU, however, showed fewer signs of disease than control animals after challenge (Table 1). At this intermediate dose, dl27gD2 protected somewhat better than dl27lacZ1 from disease and also prolonged the time of death (dl27gD2, $P = 0.0088$; dl27, $P = 0.0088$; dl27lacZ1, $P = 0.0309$) but time to death in the dl27gD2-immunized group was less than that of the 5BlacZ group (Fig. 5B; $P = 0.0078$). At the highest dose of immunizing virus, $10^6$ PFU, the difference between the levels of protection in animals receiving dl27lacZ1 or dl27gD2 and those receiving 5BlacZ was equally pronounced (Table 1; 5BlacZ, $P = 0.046$ compared with control). Moreover, the only group in which all the ani-

**TABLE 1**

<table>
<thead>
<tr>
<th>Immunizing virus</th>
<th>Dose of immunizing virus (PFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^4$</td>
</tr>
<tr>
<td>5BlacZ</td>
<td>4.1 ± 0.4$^a$</td>
</tr>
<tr>
<td>dl27gD2</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>dl27lacZ</td>
<td>4.1 ± 0.4</td>
</tr>
</tbody>
</table>

$^a$ Mean score ± SEM of disease for groups of mice ($N = 5$) assessed on a 5-point scale (see Methods and Materials).
and control groups was magnified at the $10^6$ PFU dose (Fig. 3C; $P < 0.05$ for all three immunization groups on days 2 through 5). Mice immunized with 5BlacZ showed the greatest overall reduction in titer ($P = 0.0172$), and 5BlacZ was the only immunizing virus for which a dose-dependent reduction in acute replication of challenge virus was clearly evident. Levels of virus shed by mice immunized with dl27gD2 or dl27lacZ1 were comparable at all doses and times postchallenge. Thus, decreased replication of challenge virus was dependent on both the vaccine virus strain and its dose.

**DISCUSSION**

The apparent efficacy of HSV vaccine candidates in guinea pig genital models and in mouse ic models contrasts with their limited efficacy in clinical trials, as reviewed in the Introduction. This has raised concern in the herpes vaccine field that an appropriate animal model for testing HSV vaccines may not be available (Mascola, 1999). We have shown in the current study that a mouse genital infection model of HSV-2 infection has some of the features that one might postulate to be essential features of a good model, i.e., better efficacy of live viral vaccine than inactivated vaccine, levels of protection dependent on dose of vaccine, the ability to detect quantitative differences between different vaccine constructs, and the ability to show improved protection against HSV-2 infection with HSV-2 vaccine strains compared with HSV-1 strains. Obviously, the final proof that the mouse genital system is a good animal model requires the proof that efficacious vaccines in this system induce protective immunity in humans.

**Mouse genital infection model**

The mouse model that we have used is a modification of that developed by Parr et al. (1994), who utilized intravaginal infection of Depo-Provera-treated BALB/c mice with HSV-2 strain 333. Hormone treatment renders young adult female mice susceptible to HSV-2 infection by the vaginal route, and we have found that a second injection of Depo-Provera increases their sensitivity. We have utilized the HSV-2 G strain, a clinical isolate passed a limited number of times in human cells (Ejercito et al., 1968), as our challenge strain in this system. Five to 50 LD$_{50}$ doses for the challenge infection are used to push the limits of protection and have demonstrated the capacity of HSV-2 replication-defective mutant viruses to protect against lethal HSV-2 genital challenge (Da Costa et al., 1999; Morrison et al., 1998). Although the hormone treatment increases the susceptibility of the mice to HSV-2 infection, this may reduce the similarity to the human situation.

**Comparing live versus inactivated vaccine**

In the guinea pig model, glycoprotein subunit vaccines consisting of gD-2, gB-2, or gB-2 plus gD-2 (Stanberry et al., 1987, Stanberry et al., 1989a, 1989b) elicit strong glycoprotein-specific antibody responses and have provided solid protection against HSV-2 genital challenge. Furthermore, β-propiolactone-inactivated vaccine virus has shown strong protective efficacy in the guinea pig model (Da Costa et al., 1997; McLean et al., 1994). Nevertheless, subunit vaccines have not been efficacious in clinical trials, leading to the conclusion that strong cell-mediated immunity must be induced in addition to neutralizing antibodies for protection in humans (Mascola, 1999).

In the mouse genital infection system, we have demonstrated that live viral vaccine induces complete protection against lethal challenge virus infection while UV-inactivated virus vaccine provides only partial protection. Immunization with live 5BlacZ virus gave statistically significant decreases in viral shedding, disease, and mortality compared to the control cell lysate, but UV-inactivated 5BlacZ virus did not induce statistically significant changes in any of these three parameters.

**Discerning quantitative and qualitative responses**

An important feature of any vaccine assay system is that the level of immunity induced be proportional to the dose of the vaccine. Over the range of doses tested in this study, the level of protective immunity generated against challenge virus shedding, disease, and mortality was dependent on the dose of vaccine virus. In fact, the system was exquisitely sensitive to immunizing dose, with the spectrum between complete protection and uniform disease and death covered by a 100-fold range of virus inocula.

A second important issue in herpes simplex vaccination is the capacity to distinguish the optimal virus strain for protection against HSV-2 at its preferential site of infection. Given that prior infection with HSV-1 in humans lowers the probability of genital infection with HSV-2 only two- to threefold (Bryson et al., 1993; Corey et al., 1983; Mertz et al., 1992), whereas previous infection with a heterologous strain of HSV-2 considerably lowers the probability of reinfection (Lakeman et al., 1986; Schmidt et al., 1984), it seems likely that homotypic immunization strategies would be most successful in protecting the genital tract. Previous studies in guinea pigs have shown that HSV-1 vaccines can provide a high level of protection against HSV-2 genital infection (McLean et al., 1994). Our study clearly indicates a difference between HSV-2 (5BlacZ) and HSV-1 (dl27lacZ1) vaccine strains that cannot be overcome by provision of gD-2 in the HSV-1 genetic background (dl27gD2). That this system can be used to rank different viral vaccines for their efficacy is its most important feature.
Clinical trials utilizing gD-2 (Straus et al., 1994) or gD-2 plus gB-2 (Corey et al., 1999; Straus et al., 1994) have shown that the immunity induced by these glycoproteins does not provide adequate protection against HSV-2 infection. Our results indicate that HSV-2 gD in the context of a live HSV-1 vaccine also is not sufficient to protect against HSV-2 genital infection and disease, although protection is superior to heterotypic HSV-1 immunization. The strongest protection is obtained with an HSV-2 replication-defective mutant. It is not clear how many other than HSV-2 gene products gD-2 are needed to provide the breadth of immunogen necessary for protective immunity, or which gene products are most important immunologically, but our results clearly indicate that multiple gene products will be necessary.

The currently available information indicates that effective immunization against genital HSV-2 infection requires the induction of cellular immunity by way of live viral vaccination, ideally using a broad range of HSV-2 antigens. The murine genital infection model system employed in this study also indicated an apparent requirement for these features to achieve protective immunity. Further studies are needed to determine whether the differences between the murine system and guinea pig system reflect qualitative differences in sensitivity or are due to the lower doses of virus used in the mouse model, the strains of virus used in the different systems, or differences in the immunology of the two species. In any event, our results indicate that this murine system or refined versions of other animal infection models may be useful in ranking different genital herpes vaccine candidates for their protective capacity against HSV-2.

MATERIALS AND METHODS

Cells and viruses

V27 (Rice and Knipe, 1990) and S2 cells (Gao and Knipe, 1989) were used to propagate ICP27 and ICP8 mutant viruses, respectively. The HSV-1 ICP27 mutant virus, dl27lacZ1 (Rice and Knipe, 1990), and the HSV-2 ICP8 mutant virus, 5BlacZ (Da Costa et al., 1997), have been described previously. HSV-2 strain G (Ejercito et al., 1997) was used as the challenge virus. The construction of cell lines that complement ICP8 or ICP27 mutant viruses. One such clone of cells, named V827, was selected and used for all further studies.

Plasmids

The HSV-2 strain 186 syn1 (gD-2) gene was isolated as an Xho fragment from plasmid pEH43 (Knipe and Spang, 1982). Plasmid pPs27d-1 (Rice and Knipe, 1990), which lacks the ICP27 ORF, but has flanking HSV sequences, was linearized with BamHI and blunt ended. A unique Xho site was created at this junction by linker addition (5'-CCGCTCGAGCGG-3'; New England Biolabs). The gD-2 gene was cloned into this site and a single plasmid having the gD-2 ORF in the same orientation as that of ICP27 was selected and named pPs27dXho-gD2.

Construction of d27gD2 recombinant virus

An ICP27 mutant virus expressing gD-2 from the U54 (ICP27) locus was isolated by cotransfecting dl27lacZ1 genomic viral DNA and plasmid pPS27d-Xho-gD2 into V27 cells using a protocol described previously (Da Costa et al., 1997). The plasmid was linearized outside the HSV sequences to facilitate recombination. Progeny viruses were harvested and replated on V27 cells under an X-Gal overlay. White plaques representing possible gD-2 recombinant viruses were selected against a background of blue parental viruses. Several independently isolated white plaques were purified at least thrice each and the presence of the gD-2 gene was confirmed by Southern hybridization (data not shown). One such virus was called dl27gD2 (Fig. 1) and used in further experiments.

Immunoblot analysis of gD-1 and gD-2 expression

The capacity of the mutant viruses to express gD was examined by immunoblot assay. Infected-cell lysates were subjected to SDS-PAGE, electroblotted onto nitrocellulose, and probed for gD-1 or gD-2 expression. Rabbit polyclonal sera R-45 (kindly provided by Dr. G. Cohen and Dr. R. Eisenberg) and anti-gD2T M13 (kindly provided by Dr. D. C. Johnson) were used as the primary antibodies to detect gD-1 and gD-2, respectively. Secondary antibodies and detection reagents were as described previously (Da Costa et al., 1997).
Animal studies

Female BALB/c mice, 6 weeks of age, were obtained from Taconic (Germantown, NY). Mice received food and water ad libitum and were rested for 1 week before use. Housing of animals and all protocols and procedures were approved by institutional guidelines.

Mice were immunized with $10^6$ PFU of replication-defective virus partially purified from supernatants of infected cultures, with an equivalent amount of virus that had been UV-inactivated (Morrison and Knipe, 1994) or with control supernatants diluted in sterile 0.9% saline. In a separate experiment, animals were immunized once by sc injection in the right rear flank near the base of the tail. Animals received $20 \mu l$ of $10^4$, $10^5$, or $10^6$ PFU of viral lysate or the $10^6$ equivalent amount of control lysate diluted in sterile 0.9% saline (Sigma, St. Louis, MO). All animals were challenged at 4 weeks after immunization.

At 1 week and again at 1 day prior to challenge, animals received 3 mg of medroxyprogesterone (Depo-Provera, Upjohn) in a volume of 0.1 ml delivered by sc injection into the neck ruff. All animals were swabbed with a type 1 Calgiswab (Spectrum) and then challenged with $5 \times 10^4$–$5 \times 10^6$ PFU of HSV-2 strain G (approximately 5 to 50 LD$_{50}$) by the i.vag. route. Levels of virus shed from the genital epithelium were determined by swabbing the vaginal vault as previously described (Morrison and Knipe, 1997). Animals also were examined daily for signs of HSV disease, which was assessed on a 5-point scale (Morrison et al., 1998).

Statistics

Acute replication titers were compared on individual days by Student’s t test and over time postchallenge by area under the curve. The statistical significance of difference in proportion surviving was determined by the Fisher exact test and of difference in survival curves by the log rank test. Disease scores were compared by the Kruskall–Wallis nonparametric method.

ACKNOWLEDGMENTS

This work was supported by NIH Grant CA26345 to D.M.K. and CA75052 to L.A.M. We thank K. Hartman and L. Zhu for expert technical assistance and D. Garber for assistance with some of the statistical analysis.

REFERENCES


