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Effects of Herpes Simplex Virus Type 2 Glycoprotein Vaccines and CLDC Adjuvant on Genital Herpes Infection in the Guinea Pig

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Abstract

Genital herpes simplex virus (HSV) infections are common but results from vaccine trials with HSV-2 glycoprotein D (gD) have been disappointing. We therefore compared a similar HSV gD2 vaccine, to a further truncated gD2 vaccine, to a vaccine with gD2 plus gB2 and gH2/gL2 and to a vaccine with only gB2 and gH2/gL2 in a guinea pig model of genital herpes. All vaccines were administered with cationic liposome-DNA complexes (CLDC) as an adjuvant. All vaccines significantly decreased the severity of acute genital disease and vaginal virus replication compared to the placebo group. The majority of animals in all groups developed at least one episode of recurrent disease but the frequency of recurrent disease was significantly reduced by each vaccine compared to placebo. No vaccine was significantly more protective than gD2 alone for any of the parameters described above. No vaccine decreased recurrent virus shedding. When protection against acute infection of dorsal root ganglia and the spinal cord was evaluated all vaccines decreased the per cent of animal with detectable virus and the quantity of virus but again no vaccine was significantly more protective than another. Improvements in HSV-2 vaccines may require inclusion of more T cell targets, more potent adjuvants or live virus vaccines.

Keywords

Herpes simplex; HSV vaccine; HSV glycoproteins; guinea pig; genital herpes; CLDC adjuvant

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Introduction

Genital herpes infections are common [1] and can increase the risk of HIV infection [2]. Therefore herpes simplex virus type 2 (HSV-2) is a major target for vaccine development. Recently two vaccines were evaluated: one, an HSV-2 glycoprotein D (gD2) vaccine combined with 3-deacylated monophosphoryl lipid A (MPL) and alum and the other a gD2 with gB2 combined with MF59. The MPL adjuvanted vaccine reduced primary disease by approximately 70% in HSV seronegative women who had HSV-2 infected partners but had no effect in men or HSV-1 seropositive women [3]. The MF59 adjuvanted vaccine did not appear to be effective in a study evaluating the vaccine in men and women who were partners of HSV-2 infected individuals and in STD clinics [4].

In order to improve upon these results it may be necessary to include other HSV-2 antigens and/or more effective adjuvants. The ability to induce a potent immune response which stimulates both neutralizing antibody and T cell responses may be especially important for a herpes vaccine (reviewed in [5-6]). This approach identified glycoproteins gD, gB, gH/gL, as possible targets [7-9]. These four HSV glycoproteins are essential for entry of both HSV-1 and HSV-2 (reviewed in [10]). gD is a receptor binding protein, while gB and the gH/gL complex constitute the core fusion machinery that is needed to release the capsid inside the cell to begin replication. The core fusion machinery is triggered into a fusogenic state only after gD engages its cell receptor. A successful vaccine should target at least one key entry step and may be more protective if it targets several steps. Although both gD and gB antigens have been evaluated in guinea pig HSV-2 vaccination studies [11-14], the gH/gL antigens have only been evaluated in mice and only against HSV-1[7]. Previous studies have suggested that a combination glycoprotein vaccine is more effective than vaccination with individual glycoproteins [15]. We therefore compared the gD2 (306) vaccine previously evaluated, truncated at aa 306 [16] with a further truncated (at aa 285) gD2 (285), a gD2 + gB2 + gH2/gL2 vaccine and a gB2 + gH2/gL2. We believe this is the first evaluation of the latter two combination vaccines.

We also chose to evaluate a further truncated gD2 vaccine (gD2 (285)) because it was possible that this form of gD2 would induce more potent immune responses as we have previously shown that both gD1(285t) and gD2(285t) have a much higher affinity for receptors than gD(306t) [17-18]. There are two major cellular receptors, which act as mediators for entry of HSV-1 and -2. These are Nectin-1, a cell adhesion molecule, and HVEM, which is a tumor necrosis factor family member [19] whose function is key in regulation of the immune system [20]. HSV gD binds to either of the receptors to initiate the cascade of events leading to fusion of the virion envelope with a cellular membrane. Blocking of virion gD binding to receptor by antibodies is presumably crucial in developing HSV vaccines. The affinity of binding of gD2 (306t) for Nectin-1 and HVEM is 1.5 to 3.2×10^{-6} [21] while truncating gD to residue 285 resulted in a 100 fold enhancement in affinity to Nectin-1 and HVEM and a concomitant increase in the ability of gD285 protein over that of gD306 to block virus entry [22-23]. Ultrastructural studies, in fact, showed that residues between 285 and 306 do indeed obscure both receptor-binding sites [24]. Furthermore, gD interferes with binding of HVEM to its immunological ligands with gD285 being more active than gD306 [25]. Studies demonstrated that targeting of gD to the BTLA (an HVEM ligand) binding site of HVEM augments the immunogenicity of vaccines [20]. Thus, we now know from immunological, biochemical and ultrastructural studies that when residues between amino acids 285 and 306 are removed receptor-binding sites are more accessible to receptors. We therefore hypothesized that antibodies to these sites would be more effective at blocking receptor binding when these same residues are removed. Thus, gD285 might be a more effective immunogen than gD306.

We elected to evaluate these vaccine candidates with Cationic Lipid DNA Complexes (CLDC) as the adjuvant as we had previously shown that this adjuvant appeared to be more effective than MPL/Alum, the adjuvant used in recent clinical trials of a gD2 vaccine [3], when evaluated in mouse and guinea pig models of genital HSV-2 infection [16,26]. The cationic liposomes facilitate endocytosis and direct delivery of the plasmid DNA to the endosomal compartment thus increasing binding of nucleic acids to endosomal toll-like receptors [27]. The adjuvant activity of CLDC is mainly due to a stimulation of the innate immune system by nonmethylated CpG motifs within the plasmid which stimulate cells to produce IFN γ and IL-12 [28-29]. Because the CLDC adjuvant induces both strong B and T cell, including CD4 and CD8 T cell responses, we expected that a T cell response to the glycoproteins would enhance the protection provided by neutralizing antibody [28-32]. The addition of CLDC has recently been shown to increase both CD4 and CD8 T cell responses to an influenza vaccine and improved protection to drifted strains [33]. The induction of CD8 cells appeared to be responsible for cross protection to heterologous subtypes [34].

Methods

Animals

Female Hartley guinea pigs (250-350 g) were obtained from Charles River Breeding Laboratories (Wilmington, MA) and housed under AAALAC approved conditions at Cincinnati Children's Hospital Medical Center.

Vaccines

All vaccines were prepared by R. Eisenberg and G. Cohen (University of Pennsylvania) from Sf9 (*Spodoptera frugiperda*) cells infected with a recombinant baculovirus expressing gD2 as previously described [35]. Briefly, soluble gD2 (306) and gD2 (285) were purified from baculovirus-infected insect cells (Sf9) as previously described (25). The gD2 (306) vaccine is truncated at the transmembrane domain (aa 1-306) and is the same vaccine reported previously [16]. The gD2 (285) vaccine is further truncated (aa 1-285). The truncated proteins were purified from supernatants of baculovirus-infected cells grown in suspension. For each protein, the clarified and dialyzed medium was passed over a column of MAb DL6 coupled to Sepharose 4B, washed with 0.1 M Tris-0.15 M saline, pH 7.5 (TS), eluted with 0.1 M ethanolamine, concentrated by using a YM3 membrane (Amicon), and dialyzed against phosphate-buffered saline (PBS). Similarly, soluble gB2 (720t) was purified from baculovirus-infected Sf9 cells by use of a SS10 [36] immunosorbent column in essentially the same fashion as outlined above for gD. To make the soluble gH2t/gL2 complex, we used the FastBac Dual system (Invitrogen) to construct a single baculovirus recombinant that expressed both gH2 (truncated just before the transmembrane region at residue 803) and full-length gL2. A six-His tag on the C terminus of gH2t facilitated purification of the complex by use of Ni-nitrilotriacetic acid resin and elution with imidazole as previously described [37]. The source for all constructs was HSV-2 strain 333.

Adjuvants

CLDC (JVRS-100, Juvaris BioTherapeutics, Inc., Burlingame, CA) was provided as a white, lyophilized powder manufactured from plasmid DNA complexed with liposomes. The plasmid (pMB75.6) was 4242 base-pairs in length and was in a Tris-HCl buffer. Liposomes were prepared from the cationic lipid DOTIM (1-[2-(oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolium chloride) and the neutral lipid, cholesterol. The plasmid DNA and liposome intermediates were each diluted with lactose and then complexed under aseptic conditions, to form the formulated drug substance. The formulated drug substance was filled in vials and lyophilized to produce the drug product. The lyophilized CLDC drug product was reconstituted in sterile water for injection. After reconstitution, the final drug

product was a colloidal dispersion of 0.3 mg/ml DNA, 1.88 mg/ml DOTIM and 1.05 mg/ml cholesterol at pH 7 containing 1.4 mM Tris-HCl and 10% w/v lactose [32].

Experimental design

For evaluation of the clinical and virologic effects of prophylactic vaccination, sixty guinea pigs were randomized into five groups (N=12/group): Group 1, no vaccine or adjuvant (received 10% sucrose); Group 2, gD2 (306) + CLDC; Group 3, gD2 (285) + CLDC; Group 3, gD2 (285) + gB2 + gH2/gL2 + CLDC; Group 5, gB2+gH2/gL2 + CLDC. Animals were immunized on days 49 and 21 days prior to viral inoculation. Each 500 µl dose was administered by subcutaneous injection at 2 separate sites on the dorsum.

One day before viral challenge, animals were bled by toenail clip and the serum stored at -20°C. Animals were inoculated with virus by rupturing the vaginal closure membrane with a moistened calcium alginate tipped swab (Calgiswab #3, Spectrum Labs., Los Angeles, CA) and instilling 0.1 ml of a virus suspension containing 1×10^6 plaque forming units (pfu) of HSV-2 strain MS into the vaginal vault. Swab samples of cervicovaginal secretions were collected on days 2, 5 and 8 post inoculation (PI) and stored frozen (-80°C) until assayed for virus on rabbit kidney cells grown in BME (Gibco-Invitrogen) and 10% FBS (Hyclone, Thermo Fisher Scientific) as described previously [12].

Guinea pigs were evaluated daily and primary genital skin disease quantified using a lesion score-scale ranging from 0 representing no disease to 4 representing severe vesiculoulcerative skin disease of the perineum [12]. Following recovery from primary infection, animals were examined daily from days 21-63 PI evidence of spontaneous recurrent herpetic lesions [12]. The number of lesion days (days on which a recurrent lesion was observed on the perineum) was recorded. Vaginal swabs were also obtained for three days during the early, middle and late part of the observation period for recurrences (d21-63, i.e., early days 21, 23, 28, middle days 40, 42, 44, and late days 58, 61, 63) to evaluate for recurrent virus shedding [16]. Swabs were stored frozen (-80°C) until they were processed for PCR analysis to determine the frequency of viral shedding into the genital tract. At the end of the study, the guinea pigs were sacrificed, and the dorsal root ganglia (DRG) were harvested aseptically. DRGs were stored frozen (-80°C) until DNA was extracted for PCR evaluation of latent virus.

In order to evaluate the effects of vaccination on acute infection of the dorsal root ganglia and spinal cord, a second experiment was performed. Forty animals were randomized equally into 4 groups (N=10/group: Group 1, placebo, Group 2, gD2 (285) + CLDC (2 doses); Group 3, gD2 (285) + gB2 + gH2/gL2 + CLDC (2 doses); Group4 gD2 (285) + gB2 + gH2/gL2 + CLDC (3 doses). Animals were inoculated and vaginal swabs were obtained on days 2 and 4 as described above. On day 5 animals were sacrificed and the DRG and Spinal cord were obtained. Virus quantifications was performed on half the specimen on the day of sacrifice using the method described for quantification of acute virus shedding while the remainder was frozen for analysis by PCR.

gD2 antibody assay

Serum antibody levels against gD2 were determined as previously described [16]. Briefly the ELISA used 1.0 µg/ml gD2 (306) (vaccine preparation) diluted in coating buffer. To create the standard curve, a pool of 5 different guinea pig sera known to contain antibody to gD2 was used and assigned a concentration of 500,000 units per ml. Two fold serial dilutions of the standard starting at a dilution of 1,000 and serial 10 fold dilutions of the guinea pig sera to be tested at dilutions from 1,000 to 100,000 were made. Biotinylated goat anti guinea pig IgG (Vector Laboratories, Burlingame, CA) was used to detect the guinea

pig antibody along with peroxidase-conjugated avidin- biotin (Vector Laboratories) and. *o*-Phenylenediamine (Sigma). The average OD values of the duplicate wells of the standard were plotted using a 4 parameter best fit method. This curve was then used to calculate the units of gD antibody using the average OD for each sample. The lower limit of the assay was 150 units. The geometric mean titer (GMT) for each group was then calculated.

Neutralizing antibody assay

To measure neutralizing antibody the serum was heat-inactivated and a series of two-fold dilutions were prepared in titration medium as previously described [38]. HSV-2 MS strain (600 pfu) was added to each dilution, incubated for one-hour, and then plated onto Vero cells. After incubation for three days, the cells were stained and evaluated against the no vaccine samples for final serum dilutions that produced a 50% reduction in the number of viral plaques. The end-point titer was calculated as the log₁₀ of the dilution.

DNA isolation and conventional HSV-2 PCR

Vaginal swabs, DRG and spinal cords were isolated from the vaccinated guinea pigs as previously described [16]. Briefly, DRG and spinal cords were homogenized on ice in 500 µl of 2% FBS BME. DNA was isolated from 200 µl of the tissue homogenates and vaginal swab media using QIAamp DNA Mini Kit (Qiagen #51306) according to manufacturer's protocol. The gB gene was amplified by PCR as previously described [16]. The initial evaluation used primer sequences: gB External Forward, 5'-CCACCGGCGCTACTTCATCT -3' and gB External Reverse, 5'-CGGATGACCGTGTCGATGTC -3' to generate a 264 bp product. If results were negative, a nested PCR assay was performed using the internal primers gB Internal Forward, 5'-CCGTCAGCACCTTCATCGA -3', and gB Internal Reverse, 5'-CGCTGGACCTCCGTGTAGTC -3' to generate a 124 bp product. A HSV-2 viral DNA control, HSV-2 Quantitated Viral DNA (Advanced Biotechnologies Inc, Columbia MD), which harbors the entire HSV-2 genome, was used to generate a standard curve and as a positive control for amplification and specificity.

Real-Time PCR

To quantify viral loads in the DRG and spinal cord samples positive by conventional PCR, the external gB-F/R primers described above were used for amplification of the 264 bp product. None of the recurrent vaginal shedding samples were positive without re-amplification. Real-time PCR was performed on a Roche Lightcycler using LightCycler® Real-Time PCR SYBR Green I fluorescent dye (Roche, Indianapolis, IN). To establish a viral DNA standard curve and to determine the limit of detection of genome copy number, a ten-fold serial dilution of control HSV-2 DNA was added to 100 ng of uninfected guinea pig brain DNA to yield a standard curve ranging from 10⁰ -10⁵ copies of viral DNA. Conditions of amplification were optimized by testing the following parameters: number of cycles, annealing and elongation time, temperature, primer concentration, and reduction of primer dimer formation. Controls included water and no DNA template. FastStart DNA Master^{PLUS} SYBR Green I reaction mix (Roche, Indianapolis, IN) containing primers (1µM) and nuclease-free water was employed for all reactions. Samples (10 µl) containing 100 ng of eluted DNA were added for a total reaction volume of 20 µl. The following cycling program was used: 95°C for 10 min. followed by 50 cycles of 10 sec at 95°C, 5 sec at 61°C and 10 sec at 72°C. The amplified gene product was verified by gel electrophoresis. Limit of detection of the quantitative assay was between 1 and 10 copies with 1 copy viral genome detected in some experiments. For statistical comparisons, negative samples were assigned a copy number of 0.5 (corresponding to a 0.7 Log₁₀ per µg of DNA).

Statistics

For comparison of means data were analyzed by ANOVA followed by a student's t test comparison. The primary comparisons were placebo to each of the vaccines with a secondary analysis comparing each vaccine to gD2 (306). Statistics were not adjusted for the multiple comparisons. Incidence data were compared by Fisher's exact test. All comparisons are two-tailed. Data presented as means and standard deviation.

Results

Acute disease and infection

We had previously shown that CLDC improved the efficacy of the gD2 (306) vaccine while CLDC alone was not protective [16], [26]. In the experiments presented here, we compared the gD2 (306) to a further truncated form of gD2 (285)) as well as to a gD2 (285) + gB2 + gH2/gL2 and to a vaccine without gD2 (gB2 + gH2/gL2) when administered with CLDC. As seen in figure 1A, all vaccines provided significant protection against genital disease compared to the placebo when administered with CLDC including vaccines with only gD2 and those without gD2 i.e., gB2 + gH2/gL2 ($p < 0.001$ for each vaccine vs. placebo). However, no vaccine provided significantly more protection than any other. No animal in the groups receiving gD2 (285) or gD2 (285) + gB2 + gH2/gL2 developed any evidence of disease. Similarly, vaccination with all the vaccines significantly ($p < 0.001$ for each vaccine vs. placebo) decreased vaginal virus replication on days 2 and 5 while no vaccinated animal shed virus on day 8 compared to 5 of 12 in the placebo group (figure 1B). Protection from infection, as defined by the absence of detectable virus on day 2, was not seen in the placebo group but was seen in 5 animals in the gD2 (306) group, 2 animals in the gD2 (285) group, 5 animals in the gD2 (285) + gB2 + gH2/gL2 group ($p < 0.05$ vs. placebo group) and 7 animals in the gB2 + gH2/gL2 group ($P < 0.005$ vs. placebo group). Thus, the gB2+gH2/gL2 vaccine was as effective in providing protection of animals from infection as the vaccine which contained gD2 (285).

Recurrent disease, latency and shedding

Recurrent disease was detected in all the animals in the control group and between 7 and 10 of the 12 animals in each vaccine group. However, immunization significantly decreased the number of days with recurrent lesions from 8.0 ± 6.5 days in the placebo group to between 1.3 ± 1.8 days and 1.8 ± 2.3 days with recurrent lesions in the vaccine groups ($p < 0.01$ for each vaccine vs. placebo) (figure 2A). Again, all vaccines reduced recurrences by a similar amount. Recurrent shedding days did not appear to be reduced by any of the vaccines during any period or overall (figure 2B). When latent virus was evaluated in the DRG, it appeared that all vaccines had reduced the number of animals with detectable latent virus from 75% in the placebo group to a detection rate of between 17 and 42% in the vaccine groups (figure 3). This difference between the placebo and the vaccine groups containing gD2 was significant comparing either the percent of animals with detectable virus or the genome copy number ($p < 0.05$). However, the difference was not significant for the gB2 + gH2/gL2 group.

Antibody response to vaccine

gD2 antibody was detected in all groups receiving gD2 (figure 4A). As shown, the antibody level induced by the truncated gD2 (285) vaccine was similar to but not greater than that induced by the gD2 (306) vaccine. Although not fully understood, a significant decrease in gD2 antibody was detected in the group that also received the other glycoproteins ($P < 0.05$). Neutralizing antibody was also induced by each vaccine (GMT 2.7- 3.2 $\log_{10}$). The neutralization titer was highest in the group receiving gB2 + gH2/gL2 but this difference

was only significant compared to the gD2 (285) group (figure 4B). The gD2 (285) vaccine did not induce a higher titer of neutralizing antibody compared to gD2 (306).

Effects of vaccination on infection of the dorsal root ganglia and spinal cord

In a separate experiment we examined the effects of immunization on acute infection of the dorsal root ganglia and spinal cord. We compared groups which received 2 doses of the gD2 (285) to 2 doses of the gD2 (285) + gB2 + gH2/gL2 as well as to a group that received 3 doses of gD2 (285) + gB2 + gH2/gL2. Reduction of virus at these sites would be expected to decrease the amount of virus available for reactivation and thus presumably affect recurrences. In this experiment, no replicating virus was detected in the spinal cord of any immunized animal while 80% of the animals in the placebo group (8/10) had detectable virus ($p < 0.001$) (data not shown). When examined by real-time PCR, 50-70% of the vaccinated animals (5-7/10) had detectable virus in the spinal cord compared to 100% of the placebo recipients (10/10) (table 1). Vaccination reduced the mean viral genome level from 3.5 ± 0.9 copies/ μ g in the placebo group to between 1.6 ± 0.8 copies and 1.6 ± 1.0 copies/ μ g in the vaccine groups ($p < 0.001$ for each compared to placebo) but there were no differences comparing vaccines or comparing 2 versus 3 immunizations. Similarly, vaccination reduced the mean number of animals with detectable viral DNA in the DRG by real time PCR from 100% of the placebo animals (10/10) to between 40-60% (4-6/10) of the animals in the vaccine groups (table 1). Vaccination reduced the mean viral genome copies in the DRG from 4.2 ± 1.2 copies/ μ g in the placebo group to between 1.4 ± 0.8 copies and 1.6 ± 0.9 copies/ μ g in the vaccine groups ($p < 0.001$ for each compared to placebo). Virus was not detected in the DRG samples by culture at this time point in any group.

Notably, in this experiment, vaccination reduced the titer of vaginal virus in all immunized groups compared to control and that 3 doses of gD2 (285) + gB2 + gH2/gL2 + CLDC further reduced the titer compared to 2 doses of this vaccine ($p < 0.05$ on day 2 and $p < 0.001$ on day 4) (data not shown). It should also be noted that immunization with 3 doses significantly increased the level of neutralizing antibody ($p < 0.001$) compared to only 2 doses of vaccine (figure 5).

Discussion

A prophylactic vaccine for genital herpes should decrease or prevent genital herpes disease, modify subsequent recurrent disease and recurrent genital HSV shedding as this is the most common source for transmission [39-40]. Because it appears that recurrences are related to the amount of latent virus [41] evaluating protection of neural tissue may be a key outcome variable to assess in evaluating HSV vaccines in animal models. A gD2 vaccine (truncated at the transmembrane domain) adjuvanted with MPL/Alum has been shown to reduce genital herpes disease but only in women who are HSV-1 seronegative [3]. The same gD2 also administered with MPL/Alum vaccine was effective in guinea pigs [13]. When a similar gD2 vaccine was evaluated with CLDC, it appeared to be more effective as an adjuvant than MPL/Alum [16]. We therefore evaluated whether the addition of gB2 and gH2/gL2 with CLDC would improve the efficacy compared to gD2 with CLDC, and whether a further truncated gD2 (285) would be more protective than the gD2 truncated at the transmembrane domain, gD2 (306). We believe this is the first evaluation of an HSV vaccine consisting of the combination of gB2 and gH2/gL2. We used the guinea pig model of genital herpes because it allows assessment of all the parameters discussed above.

These studies reported here revealed several important observations. Although all of the vaccines provided significant protection from acute disease, reduced acute virus replication and reduced recurrent disease, none of the vaccines prevented local infection (defined as recovery of infectious virus in genital secretions on day 2) in the majority of animals,

prevented all recurrent disease in the majority of animals or prevented infection of neural tissues, the source of recurrences. Importantly, none of the vaccines reduced recurrent virus shedding perhaps because they did not prevent infection of the neural tissue. This is consistent with the work of Bourne, et al., who, using a similar model, showed that the gD2 + MPL/Alum vaccine previously evaluated in humans reduced recurrent disease but not recurrent shedding [38]. However, this is in contrast to our previous study with a similar gD2 vaccine that showed reduction of recurrent disease and recurrent shedding when administered with CLDC [16]. The reasons for this are unclear; however, one significant difference is that in our current study, the vaccines were administered using two immunization injection sites whereas in the first study, the vaccines were administered using 5 injection sites. The failure of the multicomponent vaccine to improve protection was somewhat surprising as we expected that the addition of other HSV-2 proteins that induce neutralizing antibody would improve protection compared to gD2 alone. It should also be noted that the vaccine containing only gB2 and gH2/gL2 was comparable to either gD2 vaccine.

The vaccines containing gD2 all induced gD ELISA antibody in all animals and of interest, the levels of this antibody were somewhat but significantly decreased when the other glycoproteins, gB2 and gH2/gL2, were mixed with gD2 even though the same amount of gD2 protein was injected. On the other hand, the highest levels of neutralizing antibody were detected in animals that received the gB2 + gH2/gL2 vaccine. In contrast to our hypothesis that the immune response might be enhanced [20], the further truncated gD2 (285) vaccine was not able to induce higher levels of gD or neutralizing antibody as compared to the gD2 (306) version and therefore efficacy was not improved. Importantly, increasing the number of vaccinations from two to three doses of the gD2 (285) + gB2 + gH2/gL2 vaccine significantly increased the levels of neutralizing antibody detected in the guinea pigs, suggesting further enhancement is possible. However, it is important to note that the majority of the studies reported here were performed with suboptimal vaccine regimens as the primary goal was to compare vaccines which are more easily accomplished with this suboptimal scheme.

In the studies reported here, the guinea pig model of genital HSV was not able to distinguish between the protection provided by the vaccines studied despite the use of multiple end points. In the past we have seen that virus shedding was a sensitive marker for distinguishing between vaccines that were otherwise protective against acute disease [42]. However, in this study, all vaccines produced a similar reduction in virus shedding except that three doses of the multicomponent vaccine reduced virus titers even greater than two doses of the vaccine demonstrating that further reductions are possible. The inability to show improved protection with the multi-component vaccines compared to gD2 alone is somewhat disappointing as the results of studies with gD2 or with gD2 and gB2 administered with MPL/Alum or MF-59 revealed no protection [4] or only ~70% protection of seronegative women but not men [3]. Thus, there is a clear need to improve protection beyond the gD2 and MPL/Alum vaccine currently in clinical testing. Our evaluations suggests that improved protection for sub unit vaccines may require changes in dosing schemes, the addition of other non glycoprotein T cell inducing antigens, or further optimization of adjuvants. Several immunogenic T cell HSV-2 antigens have been identified, such as ICP27, ICP4, or VP22 [5,9,36,43]. Furthermore, recent studies with CLDC and influenza hemagglutinin suggest that lower concentrations of CLDC and perhaps lower antigen doses may improve the adjuvant properties and outcome of immunization (Jeff Fairman, personal communication). On the other hand, it may be necessary to use a live attenuated vaccine to induce mucosal responses and a more vigorous T cell response. For example a replication defective HSV-2 vaccine was more effective than a gD2 + MPL/Alum in a guinea pig model similar to the one used here [11].

The guinea pig model of genital herpes allows evaluations of several parameters that are important for an HSV vaccine, however it appears that the current model may not allow the distinction between potent vaccines and may over predict efficacy. The model differs from human trials in that the female animals are challenged at a time when immune responses have peaked rather than over a period of 1-2 years that was used for the recent clinical trials. Further, animals are challenged once rather than being exposed on multiple occasions, as is likely to occur in sexually active humans. We therefore suggest that future evaluations of HSV-2 vaccines be conducted by challenging animals in a manner that more closely resembles human exposure.

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Figure 1B

Figure 1.

A) Effect of vaccination on the severity of acute disease. Clinical disease was evaluated daily during the acute disease (days 1-14 following intravaginal inoculation) and the severity scored on a scale of 1-4.

B) Effect of vaccination on acute vaginal virus shedding. Vaginal swabs were obtained on days 2, 5 and 8 after virus inoculation and the amount of infectious virus quantitated by plaque assay. All error bars are STD. Each vaccine significantly reduced shedding compared to placebo but there were no significant differences comparing vaccines.

* $P < 0.001$ comparing each vaccine to placebo

Figure 2A

Figure 2B

Figure 2.

A) Effect of vaccination on subsequent recurrent disease. Animals were evaluated from days 21-63 after vaginal virus inoculation (when animals have recovered from the acute disease) for the development of recurrent lesions. The number of days with a recurrent lesion is shown. Each vaccine significantly reduced the number of days with recurrences compared to placebo but there were no significant differences comparing vaccines.

B) Effect of vaccination on recurrent vaginal virus shedding. Vaginal swabs were obtained MWF from day 22-63 after vaginal virus inoculation (when vaginal virus replication from the acute illness has resolved). Samples were evaluated on days 21, 23, 26 representing the period early after recovery, on days 40, 42, 44) representing the mid period of evaluation, and late in the evaluation period (days 58, 61 and 63). The total from all days is also shown. All error bars are STD.

* $P < 0.01$ comparing each vaccine to placebo

Figure 3.

Effect of vaccination on detection of latent HSV-2 in the DRG. Animals were sacrificed at least 65 days after HSV-2 infection and latent virus assessed by PCR.

* $P < 0.05$ comparing the number of animals with detectable virus as well as comparing the quantity of latent virus in each vaccine group to the placebo.

Figure 4A

Figure 4.

A) Geometric mean gD2 antibody titer following vaccination. Animals were bled just prior to the second vaccination and just prior to virus challenge.

B) Geometric mean neutralizing antibody titer following vaccination. Neutralizing antibody was measured on blood obtained just prior to virus challenge.

* P < 0.05 vs each other group

** P < 0.05 vs gD2 (285)

Figure 5.

Geometric mean neutralizing antibody titer

Neutralizing antibody was measured on blood obtained 30 days after the last immunization.

* P<0.05 vs 2 doses of gD2 (285) + gB2 + gH2/gL2

Table 1Effect of vaccination on initial HSV-2 replication in the spinal cord and DRG⁺.

Group	N	Spinal Cord		DRG	
		% Positive	Mean Genome Copies/ μ g	% Positive	Mean Genome Copies/ μ g
No Vaccine	10	100	3.5 $\pm$ 0.9	100	4.2 $\pm$ 1.2
gD2-T + CLDC 2 doses	10	70	1.7 $\pm$ 0.8**	50*	1.4 $\pm$ 0.8**
gD2-T + (gB+gH/gL) + CLDC 2 doses	10	50*	1.6 $\pm$ 1.0**	60	1.6 $\pm$ 0.9**
gD2-T + (gB+gH/gL) + CLDC 3 doses	10	60	1.6 $\pm$ 0.8**	40*	1.5 $\pm$ 1.0**

* P<0.05

** P<0.001

⁺ Animals were sacrificed at five days after HSV-2 infection and virus assessed by PCR.