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The HSV-1 live attenuated VC2 vaccine provides protection against HSV-2 genital infection in the guinea pig model of genital herpes

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ABSTRACT

Background: Although development of an HSV vaccine is a priority there is currently no vaccine available. The recent failure of subunit vaccines suggest that presentation of more antigens via a live attenuated vaccine may be required for protection. We therefore evaluated VC2, a live attenuated HSV vaccine, engineered to be unable to enter into neuronal axons.
Methods: VC2 pathogenesis was first evaluated in guinea pigs following intravaginal inoculation. VC2 was then evaluated as a prophylactic and therapeutic vaccine and compared protection to a gD2 vaccine adjuvanted with MPL/Alum in the guinea pig model of genital HSV-2. The guinea pig model allows evaluation of acute and recurrent disease, as well as vaginal shedding acutely and during episodes of recurrent activation.
Results: VC2 was significantly attenuated in guinea pigs compared to the wild type strain, 17syn+. It replicated poorly at the inoculation site, did not produce any genital disease and rarely infected the neural tissue. After prophylactic vaccination, the VC2 vaccine decreased the clinical severity of acute and recurrent HSV-2 disease and shedding and decreased the quantity of virus in the DRGs. When compared to gD2+MPL/Alum, VC2 was somewhat more effective especially as it relates to neural tissue infection. VC2 was not effective as a therapeutic vaccine.
Conclusion: The live attenuated prophylactic HSV vaccine, VC2, was effective in the guinea pig model of genital HSV-2. Its decreased ability to infect neural tissues provides advantages over other live attenuated vaccines.

1. Introduction

The development of an effective vaccine for genital herpes remains a priority [1–3] because it is a common infection that causes physical and emotional stress as well as increasing the risk for HIV infection [4–6] and perhaps Alzheimer's disease [7,8]. Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are the leading causes of genital ulcer disease worldwide [9,10]. Both viruses also cause neonatal herpes, a devastating disease, most often acquired from HSV infected mothers [11].

There are a number of approaches that have been taken to develop an HSV vaccine including sub-unit vaccines, peptide vaccines, live attenuated vaccines, inactivated whole virus vaccines, DNA vaccines, disabled single cycle viruses and vectored vaccines [1–3]. Most recently the leading candidate has been an HSV-2 glycoprotein D (gD2) vaccine administered with a potent adjuvant, alum/MPL [12,13]. Although the initial trials were promising, at least for effectiveness in HSV seronegative women [12], the larger trial that enrolled only seronegative women [13] showed no effect on HSV-2 infections although it surprisingly prevented HSV-1 infections and disease. This failure has led many to believe that an effective vaccine will need to present more HSV-2 antigens to the immune system [14]. Thus, there is a renewed interest in live attenuated HSV vaccines which have advantages over subunit vaccines because replication allows for the entire repertoire of virus specified antigens to be presented to the immune system. A vaccine for Varicella Zoster virus (VZV), a virus belonging to the same alphaherpesvirus subfamily as HSV, has been available in the US for more than 20 years and has resulted in dramatic decrease in disease incidence, while exhibiting an excellent safety profile [15]. The similarities between target organs and immunity induced by both viruses suggests that a live, attenuated HSV vaccine may be an effective vaccination approach [16].

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VC2 is a live attenuated HSV-1 strain [17,18]. Initially, a gK-null virus was constructed and shown to be unable to infect ganglionic neurons or establish latency after ocular infection of mice [19,20]. Next the gK-null virus was shown to provide protection against intravaginal challenge of mice with either virulent HSV-1 (McKrae) or HSV-2(G) viruses [21]. To further improve on this vaccination approach, the VC2 vaccine was developed with specific deletions within the genes coding for glycoprotein K (gK) and UL20. The VC2 virus contains a gKΔ31-68 mutation that prevents the virus from infecting ganglionic neurons after ocular infection in mice [22]. The VC2 virus replicates efficiently in cell culture including Vero cells, although plaque size is slightly reduced compared to the parent HSV-1 strain. In contrast, the gK-null virus requires replication in the complementing cell line, VK302, that expresses gK [21]. In mice VC2 produced a robust humoral and cell-mediated immune response and conferred 100% protection against lethal intravaginal challenge with either HSV-1 (McKrae) or HSV-2 (G) viruses [17,18]. In rhesus macaque VC2 elicited robust immune responses [18]. The inability of the VC2 virus to enter via fusion of the viral envelope with cellular membranes [23], may lead to significant upregulation of innate and downstream cellular immune responses to the virus that differ significantly from those produced by the wild-type parental virus HSV-1(F) [24,25].

In this report we extend these observation on the VC2 vaccine by exploring its safety and efficacy using both prophylactic and therapeutic vaccination in the guinea pig model of genital HSV-2 infection. Unlike mice, guinea pigs survive vaginal inoculation with HSV-2 and develop a latent infection that can reactivate to produce recurrent lesions or recurrent vaginal virus shedding in the absence of lesions [26]. Thus, this model more closely mimics the genital disease in humans than other small animal models and provides numerous outcomes for evaluation of vaccine efficacy.

2. Materials and Methods

2.1. Vaccines

The VC2 recombinant virus was constructed by KG Kousoulas (Louisiana State University, Baton Rouge, Louisiana) utilizing the two step double-Red recombination protocol implemented on the cloned HSV-1(F) genome [22] in a bacterial artificial chromosome (BAC) plasmid [27], as described previously [28,29]. VC2 contains the gKD31-68 deletion (37 aa; gK aa 31–68) in the amino terminus of gK as well as a deletion of the amino-terminal 19 amino acids of the UL20 virus as confirmed by next generation whole genome sequencing. No other nucleotide changes were detected comparing the parental HSV-1(F) BAC and VC2. The vaccine was diluted with DMEM for intramuscular administration.

The gD2 (3 0 6) vaccine was prepared by G. Cohen (University of Pennsylvania) from Sf9 (Spodoptera frugiperda) cells (GIBCO BRL) infected with a recombinant baculovirus expressing gD2 as previously described [30]

2.2. Adjuvants

The MPL/Alum combination contained 50 μg pg MPL (Sigma-Aldrich Corporation, St. Louis, MO) and 200 μg of Alhydrogel (2%) (Accurate Chemical and Scientific Corporation, Westbury, NY) [31].

2.3. Viruses and cells

HSV-2 strain MS (ATCC-VR540) was grown in low passage primary rabbit kidney cells and titered on rabbit kidney cell monolayers as previously described [32]. HSV-1F strain, the VC2 virus, and HSV-1 17syn + were grown in Vero cells [33].

2.4. Animals

Female Hartley guinea pigs (251–300 or 301–350 g) were obtained from Charles River Breeding Laboratories (Wilmington, MA) and housed under AAALAC approved conditions at Cincinnati Children's Hospital Medical Center. All procedures and protocols were approved by the Cincinnati Children's Hospital Research Foundation Animal Care and Use Committee.

2.5. Study design and methods

We initially evaluated the pathogenesis of the VC2 and parent F strain by vaginally inoculating 30 guinea pigs (n = 15) with $1X10^7$ PFU of either virus using the procedure described below. We evaluated primary disease daily and vaginal virus replication on days 2,4, 6 and 8. Infection of the dorsal root ganglia (DRG) was evaluated by sacrificing 2–4 animals/ group on days 4, 6 and 14 also as described below. We then extended these evaluations in a second experiment to include HSV-1 strain 17syn+. We vaginally inoculated 18 animals with VC2 and 9 animals with either F strain or 17syn+group. Evaluations were performed as indicated above with the addition of explant co-cultivation on the DRG to assess live virus recovery from the neural tissues. In the second experiment we also increased the number of animals evaluated so that 2–6 animals were sacrificed on days 4,6,14 and 28.

As previously described [34] to infect and challenge animals, a pre-moistened calcium alginate swab (Puritan Calgiswab Type 3 Guilford, ME) was used to rupture the vaginal closure membrane of each animal. Using a 1 ml slip tip syringe (BD), $1x10^6$ pfu of HSV-2 (MS strain) was instilled into the vaginal vault in a 0.1 ml suspension. To determine acute vaginal virus replication, vaginal swabs were collected on select days and titers determined on Vero cells. During primary infection, a lesion score-scale ranging from 0 representing no disease to 4 representing severe vesiculoulcerative skin disease of the perineum was used [34]. The animals were then evaluated daily from 15 to 56 days post infection (dpi) for evidence of spontaneous recurrent herpetic lesions. To assess recurrent virus shedding, animals were vaginally swabbed three times a week. The swabs were stored frozen (-80 °C), DNA extracted followed by measurement of HSV-2 DNA to determine the frequency of viral shedding into the genital tract by qPCR analysis. At the end of the study, the guinea pigs were sacrificed, and the spines were harvested and stored frozen (-80 °C) followed by dissection to obtain dorsal root ganglia (DRG).

In the prophylactic experiment, 54 Hartley guinea pigs (250–300 g) were divided into three groups (n = 18): group 1: no vaccine control, group 2: $1x10^6$ pfu VC2, and group 3: 5 μg gD2 + MPL/Alum. Animals were vaccinated by the IM route at days 63, 42, and 21 days before challenge. After intravaginal challenge with HSV-2 MS strain the animals were swabbed on 2, 4, 6, and 8 dpi to measure acute vaginal virus titers. To determine the effect of the vaccine on preventing viral infection of the neural tissue during the acute infection, the DRG were harvested and quantified by qPCR from 3 animals per group on days 4 and 6 post infection. The animals were also examined daily for recurrent lesions from 14 to 56 dpi. At the end of the study (56 dpi) the animals were sacrificed and the DRG were harvested to determine the latent viral burden of both the vaccine and challenge strains using HSV-1 and HSV-2 specific primers as described below. For evaluation of recurrent vaginal virus shedding, vaginal swabs were collected three times a week beginning at 21 dpi and continued until 56 dpi. Swabs were stored frozen until analysis by qPCR.

In the therapeutic experiment, 45 HSV-2 MS strain infected Hartley guinea pigs (300–350 g) were divided into three groups ($n = 15$): group 1: No Treatment control, group 2: $1x10^7$pfu VC2, and group 3: 5 μg gD2 + MPL/Alum. Infected animals were randomized based on weights, primary disease score, and 2 dpi vaginal titers. Infected animals were vaccinated by the IM route at days 14, 28 and 42 days after challenge. Evaluation for recurrent lesions and recurrent vaginal virus shedding were performed as described above.

2.6. qPCR of HSV-1 and HSV-2 DNA

Viral levels of HSV-1 DNA in DRGs harvested at 4, 6 and 14 dpi were determined as previously described [17]. Briefly, the DRGs were homogenized on ice in 0.5 ml (DRGs)-) of 2% FBS BME. DNA was extracted from tissue homogenate using the QIAamp DNA Mini Kit (Qiagen #51306) according to the manufacturer's protocol. The eluted DNA was quantified using a Nanodrop 2000 spectrophotometer and equal amounts of DNA were used to perform quantitative PCR using an Applied Biosystems 7500 Fast Real-Time PCR System. To maximize sensitivity, extracted DNA was evaluated at concentrations ranging from 100 to 800 ng. DNA previously purified from HSV-1 (McKrae) strain was used as a positive control and DNA previously extracted from an uninfected animal was used as a negative control. Specific primers were used to detect HSV-1 (F strain) DNA. The primers and probe were obtained from Sigma-Aldrich and the sequences were as follows:

Forward: 5′-ACG/TAC/CTG/CGG/CTC/GTG/AAG/A-3′; reverse: 5′-TCA/CCC/CCT/GCT/GGT/AG-3′; and probe: 5′-FAM-AGC/CAA/GGG/CTC/CTG/TAA/GTA/CGC/CCT-tamRA-3′.

To determine the amount of HSV-2 shed and viral levels of HSV-2 in DRGs, HSV-2 gG2 gene detection was performed by quantitative PCR. The gG2 primer and probe sequences were as follows [35]:

Forward: 5′-CGG/AGA/CAT/TCG/AGT/ACC/AGA/TC-3′; reverse: 5′-GCC/CAC/CTC/TAC/CCA/CAA/CA-3′; and probe: 5′-FAM-ACC/CAC/GTG/CAG/CTC/GCC/G-tamRA-3′.

Each PCR reaction contained 100 ng of DNA, 50 μM of each primer, 0.10 μM of FAM/tamRA fluorescent probe, and 10 μl of Taqman Gene Expression Master Mix (ABI) in a total volume of 20 μl reaction. PCR amplification of both HSV-1 and HSV-2 DNA was performed on a 7500 Fast Real-Time PCR system (ABI) using the following conditions: pre-incubation at 50 °C for 2 min and 95 °C for 10 min followed by 50 cycles consisting of a denaturation step at 95 °C for 15 s, annealing at 60 °C for 1 min, and elongation at 72 °C for 10 s. A standard curve for each virus was generated with ten-fold serial dilutions of purified HSV-1 or HSV-2 DNA (ATCC) containing 10^5-10^0 HSV-2 copies in 50 ng of uninfected guinea pig brain DNA. The limit of detection for both HSV-1 and HSV-2 was determined to be between 10^0 and 10^1 copies, with excellent linearity ($R \geq 0.98$) over 5 logs of HSV genomic DNA content.

2.7. Measurement of neutralizing antibody activity

Briefly, sera samples were obtained 21 days after each vaccination, heat inactivated and diluted two-fold (1:4–1:2048) in media containing 10% rabbit complement (Cedarlane, Burlington, NC) were mixed with HSV-2 (50–100 pfu) and incubated for 1 h at 37 °C [36]. The samples were then added to Vero monolayer and incubated at 37 °C for 1 h followed by a 1.5% methylcellulose overlay. After 3 days at 37 °C, the overlay was removed and plaques enumerated after staining with Crystal Violet (Sigma-Aldrich, St. Louis, MO). For each sample, the highest dilution producing a ≥ 50% reduction in plaques was considered the neutralizing antibody endpoint.

2.8. Statistical analysis

For comparison of the means for two groups a Student's t tests was performed using two-tailed analysis. For comparison of multiple groups, an ANOVA was initially performed and if significant differences among all the groups was noted, a Tukey's test to adjust for multiple comparison was used. Data are presented as means and standard deviations. Incidence data were compared by Fishers' exact test. A P value < 0.05 was considered significant.

3. Results

3.1. Pathogenesis of VC2

Following vaginal inoculation with VC2 or strain F, no genital lesions developed in either group. Vaginal virus was recovered from all animals but titers were significantly ($p < 0.01$,) lower on day 2 and 4 following VC2 infection and virus was only recovered on day 2 from VC2 infected animals while strain F was detected in 43% of animals on day 4 and 9% on day 6 (data not shown). Using our standard DNA concentration of 100 ng for PCR analysis, 3/15 DRGs) were positive for F strain DNA compared to 1/15 DRGs for VC2. To increase sensitivity the PCR analysis was repeated using increased DNA template concentrations. At the highest DNA concentration evaluated (800 ng), the F strain was detectable in 5/15 DRG while VC2 was detectable in 2/15 DRG samples. PCR analysis of the VC2 DRG samples was also performed at LSU in the laboratory of Dr. Kousoulas using the same primers. This analysis showed that all of the VC2 samples were negative for viral DNA.

Because of the unexpected attenuation of the F strain and the rare finding of VC2 in neural tissue, we repeated this experiment including strain 17syn+as an additional control and included explant co-cultivation to examine for live virus. Again, no animals inoculated with strain VC2 developed lesions while one animal that received F strain and most 17syn+inoculated animals developed disease (Fig. 1a). Vaginal virus replication was similar to the first experiment but virus was recovered rarely on day 4 and 6 from VC2 infected animals (Fig. 1b). Virus was not recovered by explant co-cultivation of the DRG from any VC2 or F strain inoculated animal while 50% of 17syn+animals had detectable virus in the DRG. When evaluated by PCR using the standard concentration all but two 17syn+inoculated animal had detectable virus DNA at any time and 4 of 5 were positive on days 14 or 28 while 3 of 5F strain inoculated animals had virus detected on days 14 or 28 compared to 1 of 10 VC2 infected animals (p = 0.08) (Fig. 1c) .Further the virus titers detected in the VC2 infected ganglia were somewhat lower compared to the F strain infected animals (NS after adjustment for multiple groups) (Fig. 1d)

3.2. Prophylactic vaccination

As shown in Fig. 2 the VC2 and gD2 + MPL/Alum vaccinated groups had significantly lower acute disease scores ($p < 0.0001$) when compared to the No Treatment group. No differences were observed between the two vaccinated groups (*NS*).

The remaining criteria evaluated during the acute period for each group, including the number of animals that developed acute disease, the amount of replicating virus in the vaginal vault, and the amount of acute replicating virus in the DRGs are shown in Table 1. Prophylactic vaccination with VC2 significantly reduced the number of animals with acute disease from 92% (11/12) in the No Treatment group to 33% (4/12) in the VC2 group ($p < 0.03$) similar to the gD2 group (5/12). Vaginal virus replication was significantly reduced in the VC2 vaccinated group compared to the No Treatment group ($p < 0.02$) and also when compared to the gD2 + MPL/Alum group on 2 and 4 dpi ($p < 0.03$). The viral loads in

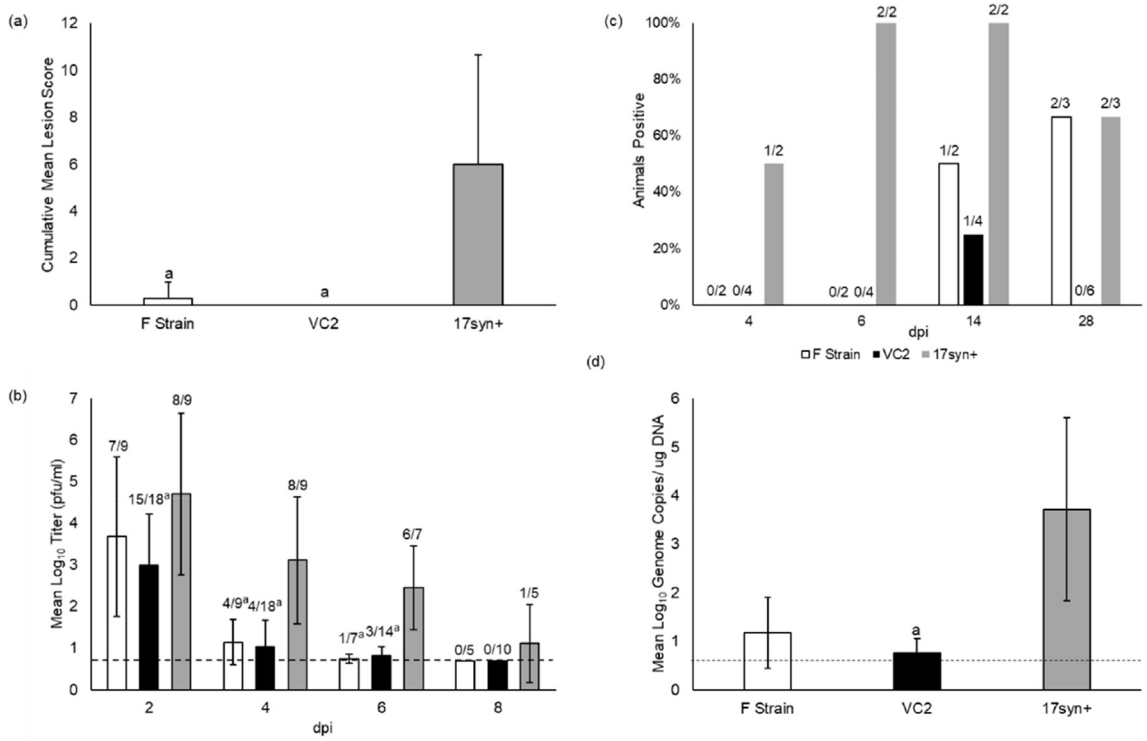

Fig. 1. Panel a shows the cumulative mean lesion scores for animals inoculated intravaginally with VC2, parent strain F and 17 syn+. Scores of 0–4 per day are combined for the 14 days after inoculation. Panel b shows the quantity of vaginally virus shed for each day while the number of animals with detectable virus is shown above each bar, panel c shows the number and percent of animals that had detectable virus in the DRG by qPCR on each day of sacrifice and panel d shows the quantity of virus detected in the DRG over all 4 days of evaluation.

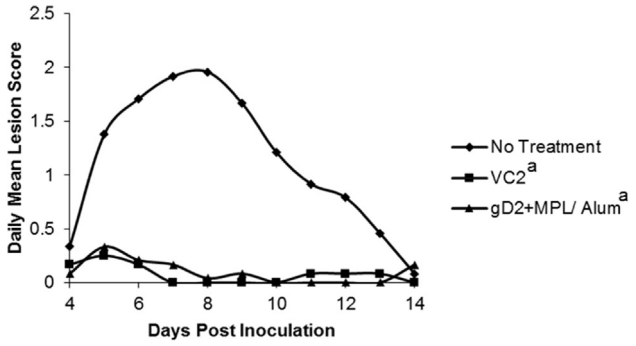

Fig. 2. Effect of vaccination on the severity of acute disease (0–14 dpi) following intravaginal challenge with 1×10^6 pfu of HSV-2 (MS Strain). The severity of the acute disease was quantified using a score-scale ranging from 0 to 4,

the DRG of VC2 animals as well as the gD2 + MPL/Alum group were also significantly less than the load in the No Treatment group ($p < 0.03$) but the differences were not significant between the two vaccinated groups.

The effects of vaccination on reduction of latent DRG infection is also an important goal of a prophylactic vaccine as it relates to recurrent disease and shedding. Latent virus was detected in 92% (11/12) of animals in the No Treatment group at 57 dpi, with a mean viral load of 2.27 ± 0.07 $\log_{10}$ genomic copies/μg DNA (Fig. 3). There was a significant (73%, p = 0.003) reduction in the number of animals with detectable latent virus in the DRG in the VC2 treated group, 3/12. Latent viral load in the DRG was also significantly decreased ($p < 0.001$) to 0.97 ± 0.50 $\log_{10}$ genomic copies/μg DNA in the VC2 group. There were no differences in the number of animals with detectable latent virus or latent viral load when comparing the two vaccinated groups (*NS*).

The reduction in neural infection was also reflected in a decrease in the number of animals with recurrent disease and the days with recurrent lesions (Fig. 4a). The VC2 vaccine group was 66% less likely to develop recurrent lesions, compared to the No Treatment group ($p \leq 0.001$) while the number of days with recurrent lesions was reduced from 8.2 ± 5.3 days in the No Treatment group to 0.9 ± 1.6 days ($p < 0.001$) and 0.8 ± 1.2 days ($p < 0.001$) for the VC2 and gD2 + MPL/ Alum groups, respectively. VC2 vaccination also decreased the number of days with recurrent

Table 1
Prophylactic Effect of VC2 on HSV-2 Acute Disease.

Group	Number of animals with Acute Disease	Vaginal Virus Titers			Viral Load in DRG	
		2 dpi	4 dpi	8dpi	4 dpi	6 dpi
No Treatment	11/12 (92%)	4.6 ± 0.9	3.3 ± 1.0	1.5 ± 1.0	5.2 ± 0.2 (3/3)	4.7 ± 0.8 (3/3)
VC2	4/12 (33%)[a]	1.8 ± 0.7[b,c]	1.7 ± 1.0[b,c]	0.7[b]	0.7[b] (0/3)	1.2 ± 0.8[b] (1/3)
gD2+MPL/Alum	5/12 (42%)[a]	2.6 ± 1.0[b]	$2.7 \pm 0.1.0$	0.7[b]	1.6 ± 0.8[b] (2/3)	2.5 ± 0.6[b] (3/3)

[a] p vs No Treatment ≤ 0.03 using Fisher's Exact test.
[b] p vs No Treatment < 0.03 using Tukey's test for multiple comparisons; 0.7 is the limit of detection and imputed as the value when no virus is detected.
[c] p vs gD2+MPL/Alum ≤ 0.02 using the Tukey's test for multiple comparisons.

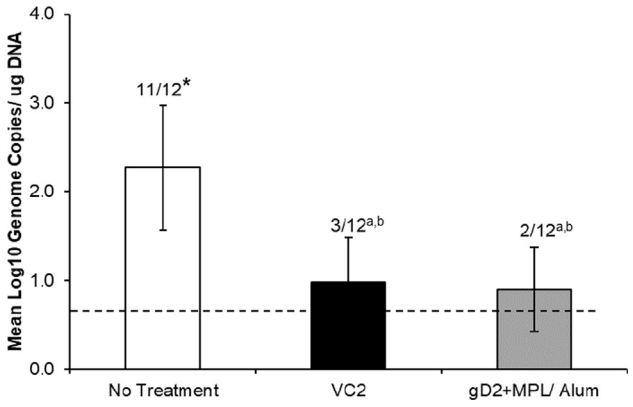

Fig. 3. Effect of vaccination on the number of animals with latent challenge virus in the DRG and the viral load of the HSV-2 MS strain challenge virus in the DRG at 56 dpi. Following evaluation of animals for recurrent disease, animals were sacrificed, the DRGs obtained and the quantity of challenge virus was quantified by PCR. The number above the bar indicated the number of animals positive for challenge virus //total number of animals in each group.

shedding compared to the No Treatment animals ($p \leq 0.2$) while gD2 + MPL/Alum had very little effect on shedding days (Fig. 4b). Animals in the VC2 group also shed less virus during each recurrence, although the difference was not significant (0.85 ± 0.12 $\log_{10}$ genomic copies/μg DNA for the VC2 group compared to 1.00 ± 0.25 $\log_{10}$ genomic copies/μg DNA for the No Treatment group, *NS*). Of note, no VC2 virus was detected in DRG of animals sacrificed at the end of the vaccine evaluation.

3.3. Neutralizing antibody

Sera samples were collected 21 days after each vaccination (before challenge) to determine the neutralizing antibody titers (Fig. 5). After two vaccinations, the neutralizing antibody titer was significantly higher in the VC2 group compared to the No Treatment group ($p < 0.001$) and more interestingly, to the gD2 + MPL/Alum vaccinated group ($p < 0.001$). After the third dose, titers were increased in both vaccine groups, with a greater increase in gD2 + MPL/Alum group. Thus, after the third

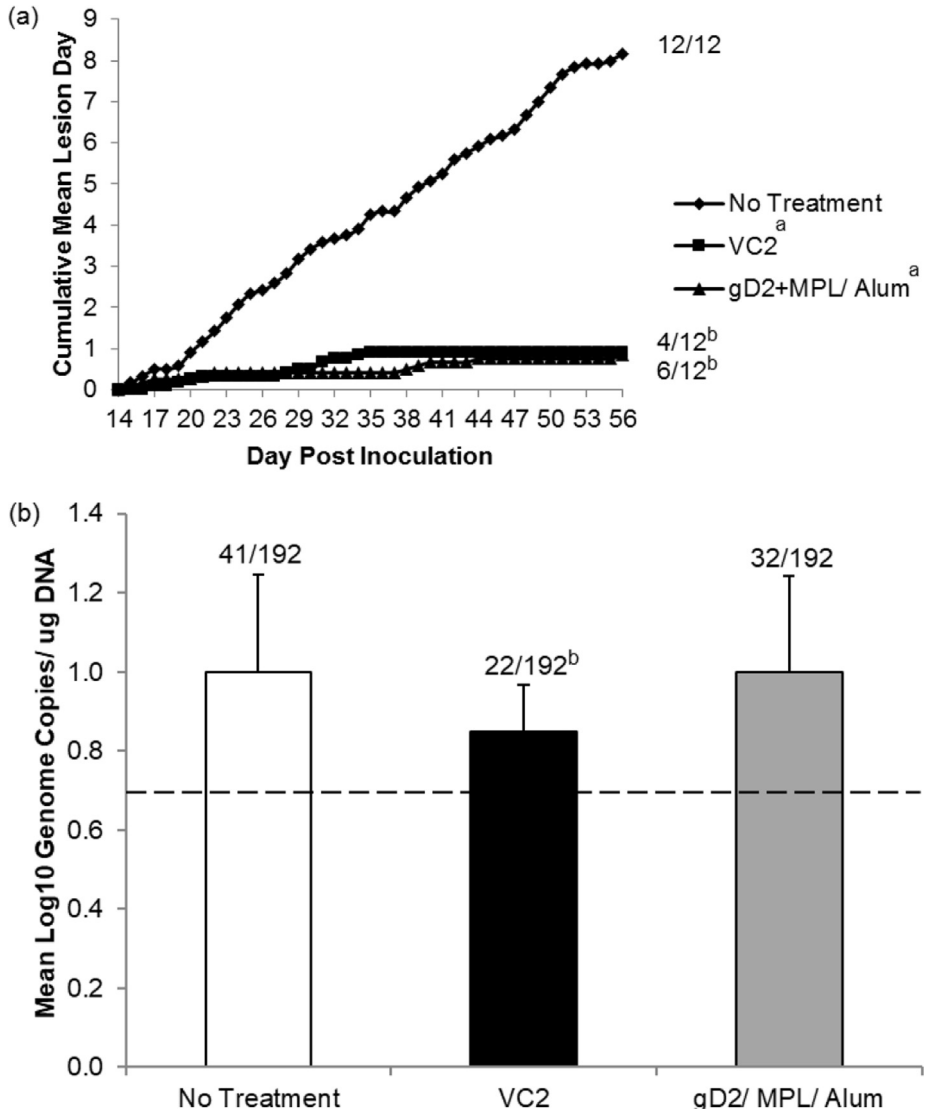

Fig. 4. Effect of vaccination on recurrent disease and recurrent shedding (days 14–56) following intravaginal challenge with HSV-2 MS strain. Animals were assessed daily for the presence of recurrent lesions and swabbed three times a week for presence of viral shedding. (a) Mean number of days with recurrent lesions. Number to the right is the number of animals with any recurrent lesions/total number of animals in each group. (b) Recurrent virus shedding detected in vaginal swabs. Number above is the number of positive days (swabs) /total number of swabs collected.

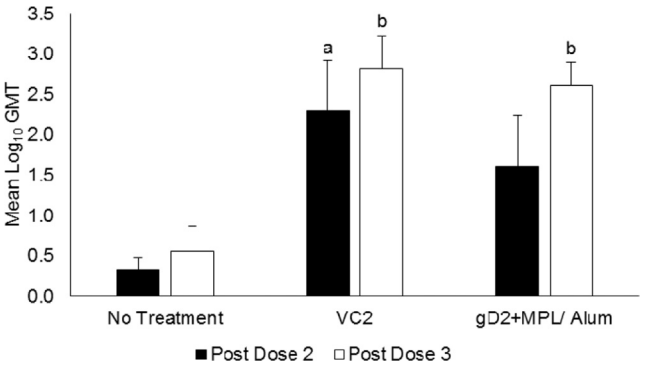

Fig. 5. HSV-2 neutralizing antibody titers obtained 21 days after each vaccination. The sera dilution that showed a 50% or greater reduction compared to the virus/complement control was used and the Log_{10} geometric mean titer presented.

vaccination neutralizing titers were not significantly different between the vaccinated groups

3.4. Therapeutic vaccination

As seen in Table 2, the effects of therapeutic vaccination were not as significant as prophylactic vaccination. Overall, the number of days with recurrent lesions was reduced in the vaccine groups compared to the No Treatment group, but the differences were not significant. There were no differences between the vaccinated groups. When the data was evaluated after each vaccination, there were also no differences in recurrent lesion days during any period. Further, the vaccines had little impact on recurrent shedding except during the period between the second and third vaccination (days 28–42) when both vaccines significantly reduced recurrent shedding days. During this period, the VC2 treated animals also shed significantly less virus ($p < 0.001$) in each shedding episode compared to the No Treatment group.

4. Discussion

Development of an effective HSV-2 vaccine remains elusive. Recent failed trials have utilized 1–2 HSV-2 proteins [12,13,37] and thus it has been suggested that a diverse repertoire of viral antigens may be needed to mount a protective immune response [3,14]. In the studies reported here, we evaluated whether a live-attenuated vaccine, VC2, would provide protection in the genital HSV-2 guinea pig model. This model allows the assessment of vaccine efficacy on acute genital disease and vaginal replication as well as recurrent disease, recurrent vaginal shedding, and latent viral load in neural tissues. Previously, the VC2 vaccine was shown to significantly protect mice from both HSV-1 and HSV-2 challenge [17].

Uniquely, the VC2 vaccine virus has deletions in the amino terminus of gK and UL20 that result in the impairment of the HSV-1 virus from establishing latency in the DRG in mice [17]. VC2 also elicited a strong immune response in mice [17] including high titer HSV-2 neutralizing antibodies and both $CD8^+$ and $CD4^+$ virus-specific T cell responses. Splenocytes from vaccinated mice produced IFNγ, TNFα, IL-4 and IL-5 but not IL-2 when stimulated with HSV specific peptides representing known or predicted $CD4^+$ and $CD8^+$ T cell epitopes. In guinea pigs, intramuscular vaccination with VC2 was shown to prime a mucosal innate immune response predisposing the adaptive expression of transcripts associated with a Th17 response which significantly contributed to antiviral immunity [25]. Significantly, in non-human primates, vaccinated animals showed no signs of disease and also developed neutralizing antibodies and T cell responses [18].

In the experiments reported here we first showed that VC2 was attenuated in guinea pigs. The virus replicated poorly at the inoculation site, did not produce any genital disease and rarely infected the neural tissue of guinea pigs inoculated intravaginally. Only low levels of virus were rarely detected early after inoculation and no virus was recovered by explant co-cultivation of the DRG of VC2 vaginally infected animals. Further, no persistent VC2 virus could be detected at day 28 after inoculation or at the end of the prophylactic vaccine experiments (day 56). Interestingly, we also found that the parent F strain was also significantly attenuated compared to HSV-1 strain 17 syn+in guinea pigs.

Our studies also showed that VC2 vaccine decreases the clinical severity of acute and recurrent HSV-2 disease in guinea pigs when provided by intramuscular vaccination. During the primary infection, animals vaccinated with VC2 had a significant decrease in clinical severity and replicating virus in the vaginal vault. Prophylactic vaccination also decreased the quantity of virus in the DRGs when compared to the No Treatment group. The ability to protect neural tissue from latent infection probably contributed to the decrease in the number of animals with recurrent lesions by 67% and the number of days with recurrent vaginal shedding by 46%. This reduction in shedding is particularly important because transmission of HSV-2 to uninfected persons is largely due to asymptomatic shedding [38,39].

The idea of using attenuated HSV strains, especially those with decreased neurovirulence goes back at least to 1986 when R. Thompson described an intertypic recombinant, RE6, that did not replicate in DRG but provided protection against challenge in mice [40]. Other more recent candidates include a mutant lacking gamma (1)34.5 gene, UL55-56, UL43.5, and the US10-12 region in the HSV-2 strain G. This virus replicated poorly in the vagina but provided protection in the guinea pig model of HSV-2. An ICP-0 deletion mutant, (HSV-2 0ΔNLS) that was more protective

Table 2
Therapeutic Effect of VC2 Vaccine.

	Group	21–28 dpi	28–42 dpi	42–62 dpi	Total
Recurrent Lesion Days	No Treatment	2.0 ± 1.2	4.6 ± 3.8	2.4 ± 1.7	9.0 ± 5.2
	VC2	2.0 ± 1.7	3.8 ± 2.7	2.5 ± 1.8	8.3 ± 4.5
	gD2 + MPL/Alum	1.2 ± 1.4	2.9 ± 2.8	2.8 ± 2.9	6.9 ± 6.6
Recurrent Shedding Days	No Treatment	31% (14/45)	28% (25/90)	5% (8/150)[b]	16% (47/285)
	VC2	42% (19/45)	8% (7/90)[a]	5% (8/150)[b]	12% (34/285)[b]
	gD2 + MPL/Alum	40% (18/45)	12% (11/90)[c]	19% (29/150)	20% (58/285)
Recurrent Shedding Quantity	No Treatment	1.2 ± 0.5	1.2 ± 0.3	0.9 ± 0.3	1.0 ± 0.2
	VC2	1.8 ± 0.5	0.8 ± 0.2[a]	0.8 ± 0.5	1.0 ± 0.2
	gD2 + MPL/Alum	1.5 ± 0.6	1.0 ± 0.3	1.1 ± 0.3	1.0 ± 0.2

[a] p vs No Treatment ≤ 0.001.
[b] p v gD2 + MPL/Alum = 0.009.
[c] p vs No Treatment = 0.02 using Fisher's exact test.

in mice than gD2 has also been described [41]. Other attenuated vaccines include a gD mutant that was attenuated but still produced some disease [42] and a gD mutant impaired for neural tropism (HSV-gD27) [43], that decreased vaginal virus replication and virus loads in the DRG compared to a gD2 vaccine given with MPL/alum in mice. Other viruses that have been developed to decrease neuronal infection include a gE deletion [44], one lacking the virion host shutoff function [45] and one with a mutation in UL24 that protected both mice and guinea pigs [46]. Perhaps most similar to the VC2 vaccine reported here is a candidate vaccine that is deleted in the pUL37 tegument protein that reportedly is not capable of spreading by retrograde axonal transport and thus, like VC2, should not reach the DRG [47]. However, the UL37-null virus apparently enters into neuronal axons and thus may more neurotoxic than the VC2 virus, which is unable to enter into neurons. Comparison of the efficacy of these vaccines is not possible because different animal models and evaluation criteria were used for each vaccine,

Using an HSV virus that does not enter neural tissues or establish persistence may have several advantages over other live attenuated HSV vaccines. Lack of persistence eliminates the possibility of recombination with any subsequent HSV strains that infect the vaccinee there by adding to its safety.

When tested as a therapeutic vaccine, i.e., one designed to decrease recurrent disease and recurrent virus shedding in those already infected with HSV, the VC2 vaccine was not effective although it did reduce recurrent shedding, at least when compared to the gD2 vaccine. This may be because live attenuated vaccines have only limited replication in hosts that are already infected and thus do not have much of an impact in improving the immune response. Because infected hosts have frequent reactivations of virus that would be similar to a live vaccine inoculation it may be difficult for the vaccine to improve the immune response unless it is able to induce responses not activated by the host's recurrent virus replication.

Overall, the live attenuated vaccine, VC2, showed promise as a prophylactic vaccine in the guinea pig model of genital herpes. The extensive safety and efficacy of another live attenuated herpes virus vaccine, varicella zoster vaccine [16] provides some reassurance that this approach is both viable and practical although hurdles exist.

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Conflicts of interest

Dr. Kousoulas has intellectual property rights to the vaccine and is a founder of is a founder of IOSBiomedical Group, INC, which owns these rights and intends to commercialize the vaccine. No other authors have a financial interest or other conflicts

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