HHS Public Access

Author manuscript

Vaccine. Author manuscript; available in PMC 2019 January 07.

Published in final edited form as:

Vaccine. 2016 January 02; 34(1): 101–109. doi:10.1016/j.vaccine.2015.10.137.

A Novel HSV-2 Subunit Vaccine Induces GLA-dependent CD4 and CD8 T Cell Responses and Protective Immunity in Mice and Guinea Pigs

Jared M. Odegard[1,7], **Patrick A. Flynn**[1], **David J. Campbell**[1,8], **Scott H. Robbins**[1,8], **Lichun Dong**[2], **Kening Wang**[3], **Jan ter Meulen**[1], **Jeffrey I. Cohen**[3], and **David M. Koelle**[2,4,5,6]

[1]Immune Design, Seattle, WA 98102

[2]Department of Medicine, University of Washington, Seattle, WA 98195

[3]Medical Virology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, 20892

[4]Department of Laboratory Medicine, University of Washington, Seattle, WA 98195

[5]Department of Global Health, University of Washington, Seattle, WA 98195

[6]Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109

[7]Current address: Benaroya Research Institute, Seattle, WA 98101

[8]Current address: MedImmune, LLC, One MedImmune Way, Gaithersburg, MD 20878

Abstract

Background/objectives: There is currently no licensed prophylactic or therapeutic vaccine for HSV-2 infection.

Methods: We developed a novel preclinical vaccine candidate, G103, consisting of three recombinantly expressed HSV-2 proteins (gD and the *UL19* and *UL25* gene products) adjuvanted with the potent synthetic TLR4 agonist glucopyranosyl lipid A (GLA) formulated in stable emulsion. The vaccine was tested for immunogenicity and efficacy in pre-clinical models for preventative and therapeutic vaccination.

Results: Vaccination of mice with G103 elicited antigen-specific binding and neutralizing antibody responses, as well as robust CD4 and CD8 effector and memory T cells. The T cell responses were further boosted by subsequent challenge with live virus. Prophylactic immunization completely protected against lethal intravaginal HSV-2 infection in mice, with only transient replication of virus in the genital mucosa and sterilizing immunity in dorsal root ganglia. Supporting the use of G103 therapeutically, the vaccine expanded both CD4 and CD8 T cells induced in mice by previous infection with HSV-2. In the guinea pig model of recurrent HSV-2 infection, therapeutic immunization with G103 was approximately 50% effective in reducing the number of lesions per animal as well as the overall lesions score.

Correspondence should be addressed to: J. ter Meulen, MD, Immune Design, 1616 East Lake Avenue.

Conclusions: Taken together, the data show that G103 is a viable candidate for development of a novel prophylactic and therapeutic HSV-2 vaccine.

Keywords

HSV-2 vaccine; GLA adjuvant; CD8 T cells; CD4 T cells

INTRODUCTION

Herpes simplex virus type 2 (HSV-2) is the leading cause of genital ulcer disease worldwide and infects more than half a billion people between the ages of 15 and 49, with new infections occurring at an estimated rate of 23 million annually (1). In addition, HSV-2 infection increases the risk of HIV acquisition 2-3 fold (2). Genital HSV-2 infection leads to latency in lumbosacral dorsal root ganglia (DRG), and subsequent virus reactivation from DRG results in clinically symptomatic recurrences or asymptomatic shedding, which poses a risk for sexual transmission between partners (3). Recurrent meningitis and neonatal herpes virus infection are serious sequels of genital herpes (4) and in the United States, health care costs associated with management of HSV-2 are among the highest for sexually transmitted diseases, second only to HIV-1 and HPV (5). While antiviral therapy reduces the duration of HSV-2 symptomatic disease, and daily suppressive therapy decreases symptomatic recurrences and asymptomatic virus shedding (6), the protection is incomplete, since antiviral therapy does not totally prevent viral shedding or eliminate latency (7). Prevention of genital HSV infection and transmission remains therefore an important target for vaccine development.

HSV-2 vaccine candidates tested in phase 3 clinical trials to date have been based on recombinantly expressed, truncated envelope glycoproteins gD2 and gB2 adjuvanted with the TLR4 agonist monophosphoryl lipid A (MPL), and alum, or alternatively with an oil-in-water emulsion. They induced neutralizing antibody and CD4 T cell responses and showed variable efficacy in the therapeutic setting, but either had no efficacy in the prophylactic setting or efficacy only in selected subgroups These studies began in the 1980s and have been reviewed (8–10). Briefly, mixed natural or bivalent recombinant gD2 plus gB2 HSV-2 glycoproteins were inactive for HSV-2 prevention (11,12), while recombinant, truncated gD2 adjuvanted with MPL, a TLR4 agonist could not be confirmed to have activity for HSV-2 prevention, but does have significant activity for HSV-1 prevention in HSV-seronegative women with binding antibody titers correlating with efficacy in a case-control investigation (13–15). Regarding therapy, monovalent or bivalent vaccines containing envelope glycoproteins have had inconsistent activity, depending on protein dose and adjuvant composition (16,17). More recently, a bivalent recombinant vaccine of gD2 and ICP4, specifically chosen as a CD4 and CD8 T cell target, has had dose-dependent and durable clinical activity in reducing genital HSV-2 lesions and shedding in a phase II clinical study (18,19). Studies conducted in mice have suggested that control of HSV-2 requires coordinated immune responses, including both antibody production and activation of $CD4^+$ and $CD8^+$ T cells depending on the details of the model system (20–25). Both cytolytic activity and interferon gamma (IFN-γ) secretion are required in rodents for complete clearance of HSV-2 from the epithelium (26). In humans, CD4+ T cells infiltrating herpetic

lesions recognize HSV-2 tegument protein and cytotoxic CD8+ T cells infiltrate and clear HSV-2 from genital herpes lesions and remain concentrated around the sensory neurons at the skin after the lesion has healed (27,28). Importantly, we have determined the frequency of peripheral cytotoxic T cells in persons with genital herpes and identified open reading frames *UL19* (encoding VP5, the major capsid protein) and *UL25* (encoding a DNA packaging protein) as prevalent targets of polyfunctional CD8 T cells (29,30). CD8 T cells specific for UL19 are detectable in PBMCs of ~46% of HSV-2 seropositive subjects and CD4 T cells are detectable in PBMC and genital mucosa of HSV-2 seropositive subjects and immune seronegative subjects (29–32). We have developed a vaccine candidate, G103, which contains 44% of full length UL19 (designated UL19ud), which contains all 3 published and 4 unpublished human CD8 epitopes, UL25 and gD. Furthermore, UL19 is highly immunogenic in preclinical models (33). UL25 contains 2 published CD8 epitopes (HLA A*0201 and B*1402) and there are two HSV-1 HLAA*2902-restricted CD8 epitopes which are sequence identical in HSV-2 (29,31,34,35). CD8 T cells specific for UL25 are detectable in PBMC of ~58% of HSV-2 seropositive subjects (29). The envelope glycoprotein D of HSV-2 (gD2) has been identified as the principal target of HSV-2 neutralizing antibodies in naturally infected humans, with subdominant contributions from gB2, gC2, and the gH2/gL2 complex (36).

G103 contains consensus sequences of the UL19 upper domain (UL19ud) identified by structural biology approaches, and of full-length UL25, both prokaryotically expressed, and the ectodomain of the glycoprotein gD2, expressed in CHO cells. The vaccine is adjuvanted with the potent synthetic TLR4 agonist glucopyranosyl lipid A (GLA) formulated in a stable oil-in-water emulsion (SE), which has been shown to prime Th1 responses and induce both antibody and T cell mediated protective immunity in multiple animal models (37–39). Importantly, GLA-SE has been safely administered to date to several hundred subjects in clinical trials as a vaccine adjuvant and shown to induce potent neutralizing antibody and CD4 responses in humans (40, S. Reed pers. comm.). Specific TLR4 agonists can differentially activate the inflammasome pathway, such that GLA should not be considered biologically equivalent to the MPL used in previous HSV-2 vaccine candidates [41]. The specific lipid in G103 also activates the inflammasome pathway [42]. gD2 is also a population-prevalent CD4 T cell antigen and contains CD8 T cell epitopes for mice and humans (34,43,44). Here we show that a vaccine containing gD, UL19, and UL25 formulated with GLA-SE adjuvant induced potent humoral and cellular immunity in mice and protected mice and guinea pigs as a prophylactic and therapeutic vaccine.

RESULTS

The development of G103 was performed in a step-wise manner, first optimizing the dose of GLA-SE adjuvant in the context of a monovalent recombinant gD2 vaccine applied in a prime-boost regimen (0, 28 days). As shown in Suppl. Figure 1A, GLA-SE and SE induced a significantly higher IgG response to gD2 compared to control, however, only GLA-SE resulted in the production of the Th1-associated isotype IgG2c, whereas the Th2-associated isotype IgG1 was GLA-independent (Suppl. Figure 1B, C). In line with the Th1-type antibody response, GLA induced gD2-specific IFN-γ producing CD4 T cells, many of which also produced TNF-α and IL-2 (Suppl. Figure 2A-D). Notably, the CD4 Th1 dose-

response curve was bell-shaped, with the peak frequency occurring at the 1-5 μg GLA dose (Suppl. Figure 2B and 2C). In mice vaccinated with gD2 plus GLA-SE long-lived gD2-specific memory CD4 T cells were detectable in the circulation 10 months after the boost (Suppl. Figure 3A). Strikingly, upon intravaginal challenge with an attenuated thymidine kinase-deficient HSV-2 (Tk^-) strain, the gD2-specific CD4 T cells expanded more than 40-fold (Suppl. Figure 3B), confirming that they were functional memory cells. Notably, memory formation was GLA-dependent, as mice primed with gD2 in SE alone did not show this response despite being challenged with live virus. Based on these data, we selected the 5 μg dose of GLA in 2% SE emulsion, as the adjuvant component of G103.

G103 induces anti-gD2 binding antibodies of the subclasses IgG1 and IgG2c, virus neutralizing antibodies, and Th1 type CD4 and CD8 T cell responses

The trivalent vaccine G103 induced similar gD2-specific IgG1 and IgG2c antibody titers as immunization with gD2 and GLA-SE (Suppl. Figure 4 A, B). We also measured serum neutralizing antibody responses in mice immunized with G103 or control vaccines. Animals immunized with unadjuvanted trivalent vaccine, or GLA-SE or the dextrose vehicle used for injection, had HSV-2 neutralizing antibody levels below the lower limit of detection. In contrast, the mean neutralizing titer amongst five mice immunized with G103 was 1:69 (range, 1:35 to 1:105), with all 5 animals responding (Suppl. Figure 4 C).

Mice given a single immunization with G103 mounted CD4 T cell responses to each antigen (Figure 1A). As recombinant protein vaccines are generally thought to preferentially target the MHC class II processing pathway, we were surprised to also observe priming of CD8 T cells specific for UL19 (Figure 1B). To confirm that these cells were functional, we immunized mice with G103, then challenged 28 days later with HSV-2 Tk^-, and found that they expanded robustly and early, with the peak memory response of antigen-specific splenic CD8 T cells reaching 2% at seven days after recall. In contrast, the virus-induced primary response in non-immunized mice was significantly lower, comparable to the response in mice immunized with G103, but not challenged (Figure 1 B, C).

Prophylactic efficacy of G103 in mice

In an exploratory experiment, conducted with an earlier bivalent UL19/gD2 prototype vaccine and a $50xLD_{50}$ inoculum of HSV-2 strain 186, we showed using 10 animals per group that GLA-SE was required for survival compared to unadjuvanted bivalent vaccine, and was associated with significantly lower acute vaginal replication and detectable HSV-2 DNA in the DRG at necropsy in just 1 of 10 survivor animals (data not shown). Based on these results showing a requirement for GLA-SE but also the unexpected survival of some animals in the unadjuvanted control group, we increased the inoculum to $100xLD_{50}$ to evaluate G103. The LD_{50} in age-matched C57BL/6 mice was found to be 688 pfu/mouse, about ½ $\log_{10}$ higher than the LD_{50} we determined in similarly aged Balb/c mice used in previous studies (45–47). In addition, acyclovir treatment was added as a comparator arm, which had previously shown to prevent mortality but not translocation of the virus to the dorsal root ganglion (33). Mice were immunized with either G103, trivalent antigen vaccine without adjuvant, GLA-SE alone, or dextrose vehicle, and challenged 28 days after the boost with HSV-2 strain 186 (Figure 2A).

G103 was completely protective, with 10/10 mice surviving and showing no evidence of lesion formation (Figure 2C,D). In contrast to the first experiment, all mice treated with placebo formulations succumbed. Further, the majority of surviving mice receiving G103 had cleared all detectable virus from the genital tract by day 5 (Figure 2B). In contrast, mice receiving trivalent antigen alone or GLA-SE alone had the same acute viral loads and mortality rates as the vehicle control group. Acyclovir, to a lesser degree than vaccine, also blunted early viral replication and protected half of the treated mice from vaginal lesion formation. Importantly, it protected all 10 treated mice from mortality (Figure 2D), allowing us to compare the amount of latent virus in the DRG in this group versus vaccinated survivors. 10/10 DRG specimens recovered from G103 treated mice 100 days after immunization were protected from latent infection, compared to only 3/9 DRG in Acyclovir treated mice (Figure 2E). Taken together, these data show that G103 induces excellent protection from both acute lytic and chronic latent HSV-2 infection.

Therapeutic efficacy of G103 in mice and guinea pigs

In HSV-2 infected patients CD4 and CD8 T cells have been implicated in the immune surveillance of the mucosal epithelium and the clearance of reactivated virus, even during subclinical reactivation (48–50). In order to model whether G103 was able to boost pre-existing virus-specific memory T cells, we infected mice with attenuated HSV-2 Tk^- virus in order to establish virus-specific memory CD8 cells and tested the ability of G103 vaccination to expand these cells 28 days later. As shown in Figure 3, therapeutic immunization with G103 induced a robust, polyfunctional recall response, with almost 2% of splenic CD8 and 4% of CD4 T cells being antigen specific. As with priming, this effect was significantly mediated by GLA-SE. To study the therapeutic efficacy of G103 in the guinea pig genital disease model of HSV-2, 30 animals were inoculated intravaginally twice with HSV-2 strain 333. 16 surviving animals with primary genital lesions were normalized by division into two groups, such that the total lesion score for each group on day 5, 7, 9, and 11 was 19.5, 25.5, 14, and 11.5 for group 1, and 19.5, 23, 15.5 and 12 for group 2. On days 14 and 35, one group was treated with GLA-SE (adjuvant only) and the second group received G103. Guinea pigs immunized with vaccine had significantly reduced number of cumulative recurrences per animal (Figure 4A) as well as reduced lesion score (Figure 4B) compared to animals that received GLA-SE alone. In a second experiment, which also employed two intravaginal infections, the frequency and severity of recurrent lesions in both the GLA-SE and the G103 groups was reduced compared to the first experiment (Figure 4C), making it difficult to interpret whether these factors were impacted by vaccination. However, the lesion scores were still reduced by vaccination with G103 compared to the GLA-SE alone in the second experiment (Figure 4D) and pooling both the first and second experiments together, the lesion scores were significantly lower in animals receiving G103 versus GLA-SE ($p=0.035$, Wilcoxon). These data indicate that G103 may reduce the severity of lesions due to recurrent HSV-2 disease in a therapeutic model.

DISCUSSION

Many HSV-2 vaccine candidates have been evaluated in animal models, including recombinant protein subunit, DNA, virus vectors with glycoprotein inserts, replication-

defective and attenuated live virus vaccines. In general, these vaccines reduced mortality and severity of disease, virus replication during acute infection, recurrent disease and recurrent genital shedding of virus. However, only a small subset of candidates achieved sterile immunity as defined by complete protection against viral replication at the site of infection, prevention of dorsal root ganglia infection and absence of recurrent genital shedding of virus or HSV DNA (51). While previous work has mostly focused on neutralizing antibody responses, T cell immunogens such as ICP4 can enhance systemic CD8+ T cell responses, and adding ICP4 to a gD2 subunit vaccine reduced the number of recurrent lesions and genital viral shedding in guinea pigs (52). We show here that adding two recently discovered targets of the human T cell response, UL19 and UL25, to gD2 and formulating the recombinant proteins as a stable emulsion with the potent TLR4 agonist GLA, induced not only potent CD4 and antibody responses, but surprisingly also primed polyfunctional CD8 T cells which could be recalled by HSV-2 infection. Two immunizations with G103 conferred full protection against mortality in HSV-2 challenged mice, resulted in only a short period of mucosal shedding, and no virus being detectable in the dorsal root ganglia. In a therapeutic setting, G103 was shown to recall CD8 T cells primed by infection with an attenuated HSV-2 strain, which may form the basis of its observed efficacy in the therapeutic guinea pig model of recurrent infection.

The present study had some limitations. We did not directly compare monovalent gD2 with the trivalent composition. While monovalent gD2 presented with optimal adjuvants or delivery can be protective in the mouse vaginal model, it has failed as an HSV-2 vaccine (9,10,47). Our goal in adding UL19 and UL25 was to incorporate CD4 and CD8 T cell antigens together with a neutralizing antibody target. While we did not include as a positive control a replication competent, attenuated HSV-2 strain such as strain 333 Tk^- (53), as others have shown that this approach leads to the establishment of detectable ganglionic latency with the vaccine strain (54). It is possible that higher HSV-2 challenge doses could have revealed less than the 100% protection from mortality and establishment of latency that we observed at 100x LD_{50}, or possibly revealed a difference between the adjuvanted subunit and live attenuated virus approaches. It was encouraging that neutralizing antibodies were detected after G103 administration, but as antibody-deficient mice are still protected from lethality after strain 333 Tk^- vaccination (55), we do not feel that quantitative comparisons in neutralizing antibody titers between various candidates have specific pathophysiologic relevance in the mouse model used in this study.

GLA-SE is a fully synthetic detoxified lipid A analogue and agonist of the TLR4 receptor. It has advanced to multiple human clinical trials and found to have an acceptable side effect profile at the 5 μg dose (40), the same level used in the preclinical models detailed herein. In controlled experiments, we showed that in the murine model, the GLA-SE adjuvant was required for high titers of binding antibodies, greatly boosted the induction of acute and long-lived CD4 responses, and also enabled the priming of CD8 T cell responses to UL19. We did not observe CD8 priming for gD2 or UL25 in the C57BL/6 mouse strain, for which $H2^b$ restricted epitopes for these proteins have not been described. The G103-primed CD8 T cells were polyfunctional, and capable of brisk expansion upon exposure of mice to replicating HSV-2. In the therapeutic context, vaccination of both HSV-2-immune and chronically infected animals with G103 greatly expanded CD8 T cells; the levels observed

were significantly higher than those detected after vaccination of naïve animals, consistent with G103-elicited re-stimulation of memory CD8 T cells primed by the infection. The contribution of CD8 and CD4 T cells to the control of HSV-2 infection is highly dependent on the model used in mice (51), and cells of both subsets localize to acute and healed human HSV-2 lesions and also chronically HSV-infected human DRG (48,56,57), making it difficult to predict which effector population might be most active in humans. The detection of both CD4 and CD8 T cells responses and a Th1-influenced antibody response after G103 administration indicate that a broad acquired immune response is elicited that may be clinically effective for prevention or treatment of HSV-2 infection.

G103 induced high antibody titers of both the IgG1 and IgG2c subclass, which is reflective of the Th1 polarized immune response that GLA stimulates in C57BL/6 mice used in this study, whereas in Balb/c mice the IgG2c gene is deleted and IgG2a antibodies are produced instead (58). Antibody subclasses may play different roles in antiviral immunity, as was shown in mouse models of HSV-1 and HSV-2 and also influenza virus infection. For example, a series of subclass switch mutants made from an anti-gD2 monoclonal antibody were examined for the ability to protect against HSV-2 challenge in Balb/c mice. IgG2a was found to be more effective than IgG1 and this correlated both with activity in antibody-dependent cell-mediated cytotoxicity and with efficacy of complement-mediated neutralization (59). In the case of influenza, immunization of Balb/c mice with hemagglutinin (HA) DNA induced mainly neutralizing antibodies of the IgG1 subclass, whereas a viral replicon particle expressing HA induced mainly non-neutralizing IgG2a antibodies that correlated with clearance of virus and increased protection against lethal influenza challenge. Furthermore, induction of both antibody isotypes as measured by ELISA was a better correlate for vaccine efficacy than neutralizing antibody titer alone (60). Similarly, monoclonal antibodies of the IgG2c subclass directed against the influenza matrix protein M2 protected C57/BL6 mice from lethal challenge, whereas IgG1 monoclonals of the same specificity did not (61). Thus, it is likely that the IgG2c antibodies induced by G103 contribute to increased protection against HSV infection via complement fixation and Fc receptor activation.

Optimally adjuvanted subunit vaccines are attractive candidates for HSV-2 vaccines and the recently demonstrated high efficacy of an adjuvanted herpes zoster subunit vaccine in a phase 3 trial validates this concept (62). Indeed, several novel vaccines have been advanced to human testing over the last few years, ranging from multivalent peptide and partial-length protein to DNA vaccines with additives to enhance HSV-2 protein expression (9,51). Demonstration of immunogenicity and antiviral activity in the mouse and guinea pig models, while not necessarily predictive of clinical success, are important milestones in the rational progression to clinical testing. G103 is therefore a logical candidate for further development based on its prophylactic and therapeutic efficacy in preclinical models.

MATERIALS AND METHODS

Ethics Statement

Immunogenicity studies performed at Immune Design were approved by the Infectious Disease Research Institute (Seattle) Institutional Animal Care and Use Committee (IDRI-

IACUC). Mouse intravaginal challenge and immunogenicity experiments performed at the University of Washington (UW) were approved by the UW-IACUC. Guinea pig intravaginal challenge experiments performed at the National Institutes of Allergy and Infectious Diseases (NIAID) were approved by the NIAID-IACUC

Vaccine antigen production and formulation

Details of the composition of G103 are given in the Supplementary Information

gD2-specific antibody ELISA and HSV-2 neutralization assay

Details of the serological assays are given in the Supplementary Information

Intracellular cytokine staining (ICS)

Details of the ICS are given in the Supplementary Information

Mouse prophylactic challenge model

Female 6-7 week old C57BL/6 mice were obtained from Charles River and acclimatized at least one week. Our standard HSV-2 vaginal model has recently been detailed (63) and is similar to that used in our earlier studies of Balb/c mice (45–47). In brief, vaccines were freshly formulated on the day of injection. Vaccinations were performed twice, 4 weeks apart, by the intramuscular route into the quadriceps bilaterally in a volume of 50 μl per side. Blood was obtained before each vaccination, before challenge, and then 27 days later from surviving mice (experiment 1 only). Mice were treated with 2 mg medroxyprogesterone as described (63). Five days later and 28 days after the second vaccination they were challenged intravaginally with wildtype HSV-2 strain 186 (kind gift of Dr. David Knipe) as described (63). Stocks had a titer of 2.99 X 10^8 pfu (plaque forming units)/ml. Preliminary experiments with the challenge stock in age- and strain-matched mice established the LD_{50} as 688 pfu. The challenge dose was 50xLD_{50} in an exploratory experiment with a gD2 only vaccine, and 10xLD_{50} in the experiment with G103. Mice were observed twice daily for 21 days and euthanized if pre-morbid. In experiment 2, some mice were treated orally with ACV (acyclovir) starting 24 hours after challenge. ACV for intravenous injection (Glaxo SmithKline) was freshly diluted daily to 1 mg/ml in autoclaved water and offered *ad libitum* until day 21. In the G103 experiment, some mouse groups were euthanized on 7 days after challenge and spleens removed aseptically for immune studies.

Vaginal swabs were collected and DNA extracted as described (63). HSV-2 DNA was detected and quantified using Taqman™ PCR with primers/probes in the glycoprotein B (*UL27*) gene (63). Each specimen was checked for inhibition. Results are reported as DNA copies per swab. Lumbosacral dorsal root ganglia (DRG) were dissected from surviving mice on day 56 after challenge (experiment 1) or between 69 and 71 days after challenge (experiment 2). Ganglia (20-24 per mouse) were pooled from each mouse for DNA extraction. Quantitative PCR was performed for both HSV-2 DNA and mouse *GAPDH.* Results are reported as HSV-2 DNA copies per 10^6 mouse cells assuming 2 copies of *GAPDH*/cell.

In experiments with immune responses as the exclusive readout, intravaginal infection were performed as above, but a non-lethal dose of 1×10^6 pfu attenuated HSV-2 Tk^- virus was employed.

Guinea pig therapeutic challenge model

Five week old female Hartley guinea pigs (Charles River Laboratories) were infected twice 9 days apart with HSV-2 strain 333 intravaginally with 4×10^4 and 1×10^5 pfu for the first and second infections, respectively. On days 5, 7, 9, and 11 after the second infection, genital lesions were graded on a scale of 0 for no lesions, 1 for erythema, 2 for single or a few small vesicles, 3 for large or fused vesicles, and 4 for ulcerated lesions (64). Guinea pigs were then paired up based on their total lesion score, one of each pair was put into Group 1 and another to Group 2 randomly; this resulted in two groups of guinea pigs with very similar severity of primary HSV-2 disease. Guinea pigs were then vaccinated on days 14 and 35 post-infection by the intramuscular route in the thigh. Vaginal lesion severity was graded by direct examination of each animal daily for 52 days after the first vaccination for total lesion score, that measures the severity of lesions ranging from 0 to 4, and for the frequency of recurrent lesions with each new recurrent lesion counted as 1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

The authors thank Dr. David Knipe, Harvard Medical School, for providing virus strain HSV-2 186, and Dr Larry Corey and Dr Carlos Paya for critical reading of the manuscript. K.W. and J.I.C. are supported by the intramural research program of the National Institute of Allergy and Infectious Diseases. This work was supported by a Cooperative Research and Development Agreement between NIAID and Immune Design. We thank Daniel Y. Li for assistance with the guinea pig experiments.

Abbreviations:

HSV-2	herpes simplex virus type 2
GLA	glycopyranosyl lipid A
UL19	major capsid protein VP5 encoded by gene UL19
Non-italicized UL25	capsid protein encoded by gene UL25
DRG	dorsal root ganglia

REFERENCES

[1]. Looker KJ, Garnett GP, Schmid GP. An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection. Bull World Health Organ 2008;86:805–12, A. [PubMed: 18949218]

[2]. Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, Hayes RJ. Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. AIDS 2006;20:73–83. [PubMed: 16327322]

[3]. Tronstein E, Johnston C, Huang M- L, Selke S, Magaret A, Warren T, et al. Genital shedding of herpes simplex virus among symptomatic and asymptomatic persons with HSV-2 infection. JAMA 2011;305:1441–9. doi:10.1001/jama.2011.420. [PubMed: 21486977]

[4]. Corey L, Wald A. Maternal and neonatal herpes simplex virus infections. N Engl J Med 2009;361:1376–85. doi:10.1056/NEJMra0807633. [PubMed: 19797284]

[5]. Owusu-Edusei K, Chesson HW, Gift TL, Tao G, Mahajan R, Ocfemia MCB, et al. The estimated direct medical cost of selected sexually transmitted infections in the United States, 2008. Sex Transm Dis 2013;40:197–201. doi:10.1097/OLQ.0b013e318285c6d2. [PubMed: 23403600]

[6]. Corey L, Wald A, Patel R, Sacks SL, Tyring SK, Warren T, et al. Once-daily valacyclovir to reduce the risk of transmission of genital herpes. N Engl J Med 2004;350:11–20. doi:10.1056/NEJMoa035144. [PubMed: 14702423]

[7]. Johnston C, Saracino M, Kuntz S, Magaret A, Selke S, Huang M- L, et al. Standard-dose and high-dose daily antiviral therapy for short episodes of genital HSV-2 reactivation: three randomised, open-label, cross-over trials. Lancet (London, England) 2012;379:641–7. doi:10.1016/S0140-6736(11)61750-9.

[8]. Ashley R, Mertz G, Clark H, Schick M, Salter D, Corey L. Humoral immune response to herpes simplex virus type 2 glycoproteins in patients receiving a glycoprotein subunit vaccine. J Virol 1985;56:475–81. [PubMed: 2997470]

[9]. Johnston C, Koelle DM, Wald A. Current status and prospects for development of an HSV vaccine. Vaccine 2014;32:1553–60. doi:10.1016/j.vaccine.2013.08.066. [PubMed: 24016811]

[10]. Kuo T, Wang C, Badakhshan T, Chilukuri S, BenMohamed L. The challenges and opportunities for the development of a T-cell epitope-based herpes simplex vaccine. Vaccine 2014;32:6733–45. doi:10.1016/j.vaccine.2014.10.002. [PubMed: 25446827]

[11]. Mertz GJ, Ashley R, Burke RL, Benedetti J, Critchlow C, Jones CC, et al. Double-blind, placebo-controlled trial of a herpes simplex virus type 2 glycoprotein vaccine in persons at high risk for genital herpes infection. J Infect Dis 1990;161:653–60. [PubMed: 2181031]

[12]. Corey L, Langenberg AG, Ashley R, Sekulovich RE, Izu AE, Douglas JM, et al. Recombinant glycoprotein vaccine for the prevention of genital HSV-2 infection: two randomized controlled trials. Chiron HSV Vaccine Study Group. JAMA 1999;282:331–40. [PubMed: 10432030]

[13]. Belshe RB, Leone PA, Bernstein DI, Wald A, Levin MJ, Stapleton JT, et al. Efficacy results of a trial of a herpes simplex vaccine. N Engl J Med 2012;366:34–43. doi:10.1056/NEJMoa1103151. [PubMed: 22216840]

[14]. Belshe RB, Heineman TC, Bernstein DI, Bellamy AR, Ewell M, van der Most R, et al. Correlate of immune protection against HSV-1 genital disease in vaccinated women. J Infect Dis 2014;209:828–36. doi:10.1093/infdis/jit651. [PubMed: 24285844]

[15]. Stanberry LR, Spruance SL, Cunningham AL, Bernstein DI, Mindel A, Sacks S, et al. Glycoprotein-D-adjuvant vaccine to prevent genital herpes. N Engl J Med 2002;347:1652–61. doi:10.1056/NEJMoa011915. [PubMed: 12444179]

[16]. Straus SE, Corey L, Burke RL, Savarese B, Barnum G, Krause PR, et al. Placebo-controlled trial of vaccination with recombinant glycoprotein D of herpes simplex virus type 2 for immunotherapy of genital herpes. Lancet (London, England) 1994;343:1460–3.

[17]. Straus SE, Wald A, Kost RG, McKenzie R, Langenberg AG, Hohman P, et al. Immunotherapy of recurrent genital herpes with recombinant herpes simplex virus type 2 glycoproteins D and B: results of a placebo-controlled vaccine trial. J Infect Dis 1997;176:1129–34. [PubMed: 9359709]

[18]. Wald A, Bernstein D, Fife K, Lee P, Tyring S, Van Wagoner N, et al. Novel Therapeutic Vaccine for Genital Herpes Reduces Genital HSV-2 Shedding. 53rd Intersci Conf Antimicrob Agents Chemother 2013:abstract G – 1458a.

[19]. Wald A, Fife L, Tyring S, Lee P, Van Wagoner N, Bernstein D, et al. Therapeutic HSV-2 vaccine (GEN003) results in durable reduction in genital lesions at 1 year, 2014.

[20]. Morrison LA, Zhu L, Thebeau LG. Vaccine-induced serum immunoglobin contributes to protection from herpes simplex virus type 2 genital infection in the presence of immune T cells. J Virol 2001;75:1195–204. doi:10.1128/JVI.75.3.1195-1204.2001. [PubMed: 11152492]

[21]. Thebeau LG, Morrison LA. Mechanism of reduced T-cell effector functions and class-switched antibody responses to herpes simplex virus type 2 in the absence of B7 costimulation. J Virol 2003;77:2426–35. [PubMed: 12551980]

[22]. Hoshino Y, Pesnicak L, Cohen JI, Straus SE. Rates of reactivation of latent herpes simplex virus from mouse trigeminal ganglia ex vivo correlate directly with viral load and inversely with number of infiltrating CD8+ T cells. J Virol 2007;81:8157–64. doi:10.1128/JVI.00474-07. [PubMed: 17522198]

[23]. Iijima N, Linehan MM, Zamora M, Butkus D, Dunn R, Kehry MR, et al. Dendritic cells and B cells maximize mucosal Th1 memory response to herpes simplex virus. J Exp Med 2008;205:3041–52. doi:10.1084/jem.20082039. [PubMed: 19047439]

[24]. Nakanishi Y, Lu B, Gerard C, Iwasaki A. CD8(+) T lymphocyte mobilization to virus-infected tissue requires CD4(+) T-cell help. Nature 2009;462:510–3. doi:10.1038/nature08511. [PubMed: 19898495]

[25]. Shin H, Iwasaki A. A vaccine strategy that protects against genital herpes by establishing local memory T cells. Nature 2012;491:463–7. doi:10.1038/nature11522. [PubMed: 23075848]

[26]. Dobbs ME, Strasser JE, Chu C- F, Chalk C, Milligan GN. Clearance of herpes simplex virus type 2 by CD8+ T cells requires gamma interferon and either perforin- or Fas-mediated cytolytic mechanisms. J Virol 2005;79:14546–54. doi:10.1128/JVI.79.23.14546-14554.2005. [PubMed: 16282454]

[27]. Koelle DM, Frank JM, Johnson ML, Kwok WW. Recognition of herpes simplex virus type 2 tegument proteins by CD4 T cells infiltrating human genital herpes lesions. J Virol 1998;72:7476–83. [PubMed: 9696844]

[28]. Zhu J, Koelle DM, Cao J, Vazquez J, Huang ML, Hladik F, et al. Virus-specific CD8+ T cells accumulate near sensory nerve endings in genital skin during subclinical HSV-2 reactivation. J Exp Med 2007;204:595–603. doi:10.1084/jem.20061792. [PubMed: 17325200]

[29]. Laing KJ, Magaret AS, Mueller DE, Zhao L, Johnston C, De Rosa SC, et al. Diversity in CD8(+) T cell function and epitope breadth among persons with genital herpes. J Clin Immunol 2010;30:703–22. doi:10.1007/s10875-010-9441-2. [PubMed: 20635156]

[30]. Hosken N, McGowan P, Meier A, Koelle DM, Sleath P, Wagener F, et al. Diversity of the CD8+ T-cell response to herpes simplex virus type 2 proteins among persons with genital herpes. J Virol 2006;80:5509–15. doi:10.1128/JVI.02659-05. [PubMed: 16699031]

[31]. Ouwendijk WJD, Laing KJ, Verjans GMGM, Koelle DM. T-cell immunity to human alphaherpesviruses. Curr Opin Virol 2013;3:452–60. doi:10.1016/j.coviro.2013.04.004. [PubMed: 23664660]

[32]. Koelle DM, Schomogyi M, McClurkan C, Reymond SN, Chen HB. CD4 T-cell responses to herpes simplex virus type 2 major capsid protein VP5: comparison with responses to tegument and envelope glycoproteins. J Virol 2000;74:11422–5. [PubMed: 11070045]

[33]. Awasthi S, Mahairas GG, Shaw CE, Huang M- L, Koelle DM, Posavad C, et al. A Dual-Modality Herpes Simplex Virus 2 Vaccine for Preventing Genital Herpes by Using Glycoprotein C and D Subunit Antigens To Induce Potent Antibody Responses and Adenovirus Vectors Containing Capsid and Tegument Proteins as T Cell Immunogens. J Virol 2015;89:8497–509. doi:10.1128/JVI.01089-15. [PubMed: 26041292]

[34]. Koelle DM, Liu Z, McClurkan CL, Cevallos RC, Vieira J, Hosken NA, et al. Immunodominance among herpes simplex virus-specific CD8 T cells expressing a tissue-specific homing receptor. Proc Natl Acad Sci U S A 2003;100:12899–904. doi:10.1073/pnas.2131705100. [PubMed: 14566059]

[35]. Jing L, Haas J, Chong TM, Bruckner JJ, Dann GC, Dong L, et al. Cross-presentation and genome-wide screening reveal candidate T cells antigens for a herpes simplex virus type 1 vaccine. J Clin Invest 2012;122:654–73. doi:10.1172/JCI60556. [PubMed: 22214845]

[36]. Cairns TM, Huang Z- Y, Whitbeck JC, Ponce de Leon M, Lou H, Wald A, et al. Dissection of the antibody response against herpes simplex virus glycoproteins in naturally infected humans. J Virol 2014;88:12612–22. doi:10.1128/JVI.01930-14. [PubMed: 25142599]

[37]. Coler RN, Bertholet S, Moutaftsi M, Guderian JA, Windish HP, Baldwin SL, et al. Development and characterization of synthetic glucopyranosyl lipid adjuvant system as a vaccine adjuvant. PLoS One 2011;6:e16333. doi:10.1371/journal.pone.0016333. [PubMed: 21298114]

[38]. Pantel A, Cheong C, Dandamudi D, Shrestha E, Mehandru S, Brane L, et al. A new synthetic TLR4 agonist, GLA, allows dendritic cells targeted with antigen to elicit Th1 T-cell immunity in vivo. Eur J Immunol 2012;42:101–9. doi:10.1002/eji.201141855. [PubMed: 22002164]

[39]. Fox CB, Haensler J. An update on safety and immunogenicity of vaccines containing emulsion-based adjuvants. Expert Rev Vaccines 2013;12:747–58. doi:10.1586/14760584.2013.811188. [PubMed: 23885820]

[40]. Treanor JJ, Essink B, Hull S, Reed S, Izikson R, Patriarca P, et al. Evaluation of safety and immunogenicity of recombinant influenza hemagglutinin (H5/Indonesia/05/2005) formulated with and without a stable oil-in-water emulsion containing glucopyranosyl-lipid A (SE+GLA) adjuvant. Vaccine 2013;31:5760–5. doi:10.1016/j.vaccine.2013.08.064. [PubMed: 24075920]

[41]. Kayagaki N, Wong MT, Stowe IB, Ramani SR, Gonzalez LC, Akashi-Takamura S, et al. Noncanonical inflammasome activation by intracellular LPS independent of TLR4. Science 2013;341:1246–9. doi:10.1126/science.1240248. [PubMed: 23887873]

[42]. Desbien AL, Reed SJ, Bailor HR, Dubois Cauwelaert N, Laurance JD, Orr MT, et al. Squalene emulsion potentiates the adjuvant activity of the TLR4 agonist, GLA, via inflammatory caspases, IL-18, and IFN-γ. Eur J Immunol 2015;45:407–17. doi:10.1002/eji.201444543. [PubMed: 25367751]

[43]. Kim M, Taylor J, Sidney J, Mikloska Z, Bodsworth N, Lagios K, et al. Immunodominant epitopes in herpes simplex virus type 2 glycoprotein D are recognized by CD4 lymphocytes from both HSV-1 and HSV-2 seropositive subjects. J Immunol 2008;181:6604–15. [PubMed: 18941251]

[44]. Muller WJ, Dong L, Vilalta A, Byrd B, Wilhelm KM, McClurkan CL, et al. Herpes simplex virus type 2 tegument proteins contain subdominant T-cell epitopes detectable in BALB/c mice after DNA immunization and infection. J Gen Virol 2009;90:1153–63. doi:10.1099/vir.0.008771-0. [PubMed: 19264627]

[45]. Dutton JL, Li B, Woo W- P, Marshak JO, Xu Y, Huang M, et al. A novel DNA vaccine technology conveying protection against a lethal herpes simplex viral challenge in mice. PLoS One 2013;8:e76407. doi:10.1371/journal.pone.0076407. [PubMed: 24098493]

[46]. Shlapobersky M, Marshak JO, Dong L, Huang M, Wei Q, Chu A, et al. Vaxfectin-adjuvanted plasmid DNA vaccine improves protection and immunogenicity in a murine model of genital herpes infection. J Gen Virol 2012;93:1305–15. doi:10.1099/vir.0.040055-0. [PubMed: 22398318]

[47]. Kask AS, Chen X, Marshak JO, Dong L, Saracino M, Chen D, et al. DNA vaccine delivery by densely-packed and short microprojection arrays to skin protects against vaginal HSV-2 challenge. Vaccine 2010;28:7483–91. doi:10.1016/j.vaccine.2010.09.014. [PubMed: 20851091]

[48]. Zhu J, Peng T, Johnston C, Phasouk K, Kask AS, Klock A, et al. Immune surveillance by CD8αα+ skin-resident T cells in human herpes virus infection. Nature 2013;497:494–7. doi:10.1038/nature12110. [PubMed: 23657257]

[49]. Posavad CM, Zhao L, Mueller DE, Stevens CE, Huang ML, Wald A, et al. Persistence of mucosal T-cell responses to herpes simplex virus type 2 in the female genital tract. Mucosal Immunol 2015;8:115–26. doi:10.1038/mi.2014.47. [PubMed: 24917455]

[50]. Johnston C, Zhu J, Jing L, Laing KJ, McClurkan CM, Klock A, et al. Virologic and immunologic evidence of multifocal genital herpes simplex virus 2 infection. J Virol 2014;88:4921–31. doi:10.1128/JVI.03285-13. [PubMed: 24554666]

[51]. Awasthi S, Friedman HM. Status of prophylactic and therapeutic genital herpes vaccines. Curr Opin Virol 2014;6:6–12. doi:10.1016/j.coviro.2014.02.006. [PubMed: 24631871]

[52]. Skoberne M, Cardin R, Lee A, Kazimirova A, Zielinski V, Garvie D, et al. An adjuvanted herpes simplex virus 2 subunit vaccine elicits a T cell response in mice and is an effective therapeutic vaccine in Guinea pigs. J Virol 2013;87:3930–42. doi:10.1128/JVI.02745-12. [PubMed: 23365421]

[53]. Stanberry LR, Kit S, Myers MG. Thymidine kinase-deficient herpes simplex virus type 2 genital infection in guinea pigs. J Virol 1985;55:322–8. [PubMed: 2991558]

[54]. Slobedman B, Efstathiou S, Simmons A. Quantitative analysis of herpes simplex virus DNA and transcriptional activity in ganglia of mice latently infected with wild-type and thymidine kinase-deficient viral strains. J Gen Virol 1994;75 (Pt 9):2469–74. doi:10.1099/0022-1317-75-9-2469.

[55]. Harandi AM, Svennerholm B, Holmgren J, Eriksson K. Differential roles of B cells and IFN-gamma-secreting CD4(+) T cells in innate and adaptive immune control of genital herpes simplex virus type 2 infection in mice. J Gen Virol 2001;82:845–53. doi: 10.1099/0022-1317-82-4-845. [PubMed: 11257190]

[56]. Zhu J, Hladik F, Woodward A, Klock A, Peng T, Johnston C, et al. Persistence of HIV-1 receptor-positive cells after HSV-2 reactivation is a potential mechanism for increased HIV-1 acquisition. Nat Med 2009;15:886–92. doi:10.1038/nm.2006. [PubMed: 19648930]

[57]. Van Velzen M, Jing L, Osterhaus ADME, Sette A, Koelle DM, Verjans GMGM. Local CD4 and CD8 T-cell reactivity to HSV-1 antigens documents broad viral protein expression and immune competence in latently infected human trigeminal ganglia. PLoS Pathog 2013;9:e1003547. doi: 10.1371/journal.ppat.1003547. [PubMed: 23966859]

[58]. Martin RM, Brady JL, Lew AM. The need for IgG2c specific antiserum when isotyping antibodies from C57BL/6 and NOD mice. J Immunol Methods 1998;212:187–92. [PubMed: 9672206]

[59]. Ishizaka ST, Piacente P, Silva J, Mishkin EM. IgG subtype is correlated with efficiency of passive protection and effector function of anti-herpes simplex virus glycoprotein D monoclonal antibodies. J Infect Dis 1995;172:1108–11. [PubMed: 7561190]

[60]. Huber VC, McKeon RM, Brackin MN, Miller LA, Keating R, Brown SA, et al. Distinct contributions of vaccine-induced immunoglobulin G1 (IgG1) and IgG2a antibodies to protective immunity against influenza. Clin Vaccine Immunol 2006;13:981–90. doi:10.1128/CVI.00156-06. [PubMed: 16960108]

[61]. Schmitz N, Beerli RR, Bauer M, Jegerlehner A, Dietmeier K, Maudrich M, et al. Universal vaccine against influenza virus: linking TLR signaling to anti-viral protection. Eur J Immunol 2012;42:863–9. doi:10.1002/eji.201041225. [PubMed: 22531913]

[62]. Lal H, Cunningham AL, Godeaux O, Chlibek R, Diez-Domingo J, Hwang S- J, et al. Efficacy of an adjuvanted herpes zoster subunit vaccine in older adults. N Engl J Med 2015;372:2087–96. doi:10.1056/NEJMoa1501184. [PubMed: 25916341]

[63]. Marshak JO, Dong L, Koelle DM. The murine intravaginal HSV-2 challenge model for investigation of DNA vaccines. Methods Mol Biol 2014;1144:305–27. doi: 10.1007/978-1-4939-0428-0_21. [PubMed: 24671693]

[64]. Stanberry LR, Bernstein DI, Burke RL, Pachl C, Myers MG. Vaccination with recombinant herpes simplex virus glycoproteins: protection against initial and recurrent genital herpes. J Infect Dis 1987;155:914–20. [PubMed: 3031173]

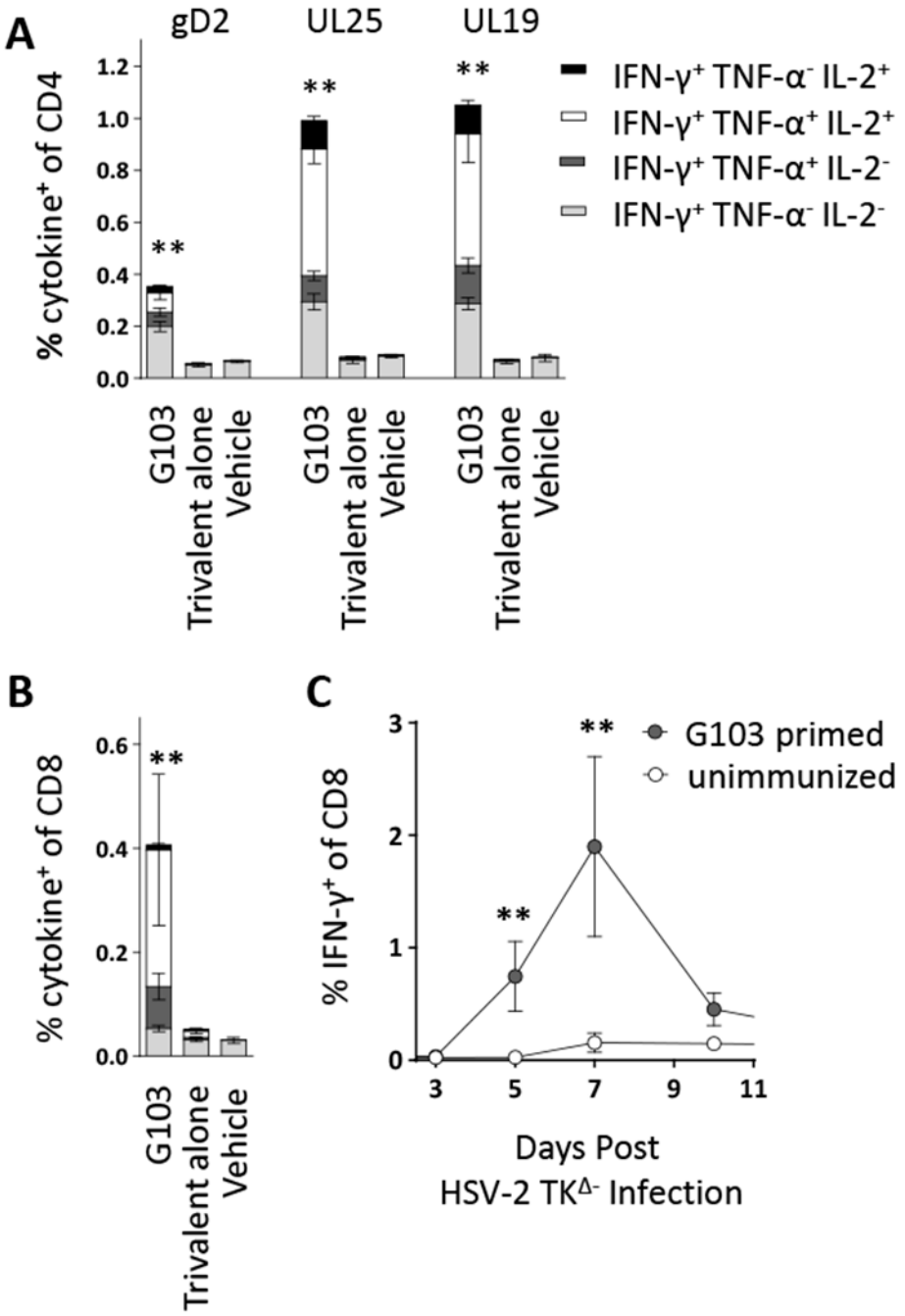

Figure 1. G103 induces T cell responses to gD2, UL19, and UL25.

A, B) Mice (n = 5 per group) were given a single immunization with either G103, or with 5μg each of recombinant gD2, UL19, and UL25 (unadjuvanted trivalent vaccine), or with vehicle only (5% dextrose). 6 days after the prime, the frequency of antigen-specific CD4 and CD8 T cells producing IFN-γ, TNF-α, and IL-2 was determined by ICS after ex vivo stimulation of splenocytes with overlapping peptide pools for each antigen. CD8 T cell responses are presented for UL19 as they were not detected for gD2 or UL25. C) To confirm the functional recall capacity of UL19-specific CD8 T cells, mice primed with G103 were

infected subcutaneously with attenuated HSV-2 Tk^- virus. The frequency of UL19-specific CD8 T cells was measured by ICS at various days post infection in immunized or unimmunized control mice. Statistically significant differences compared to control are indicated by * ($p<0.05$) or ** ($p<0.01$) (Mann-Whitney test).

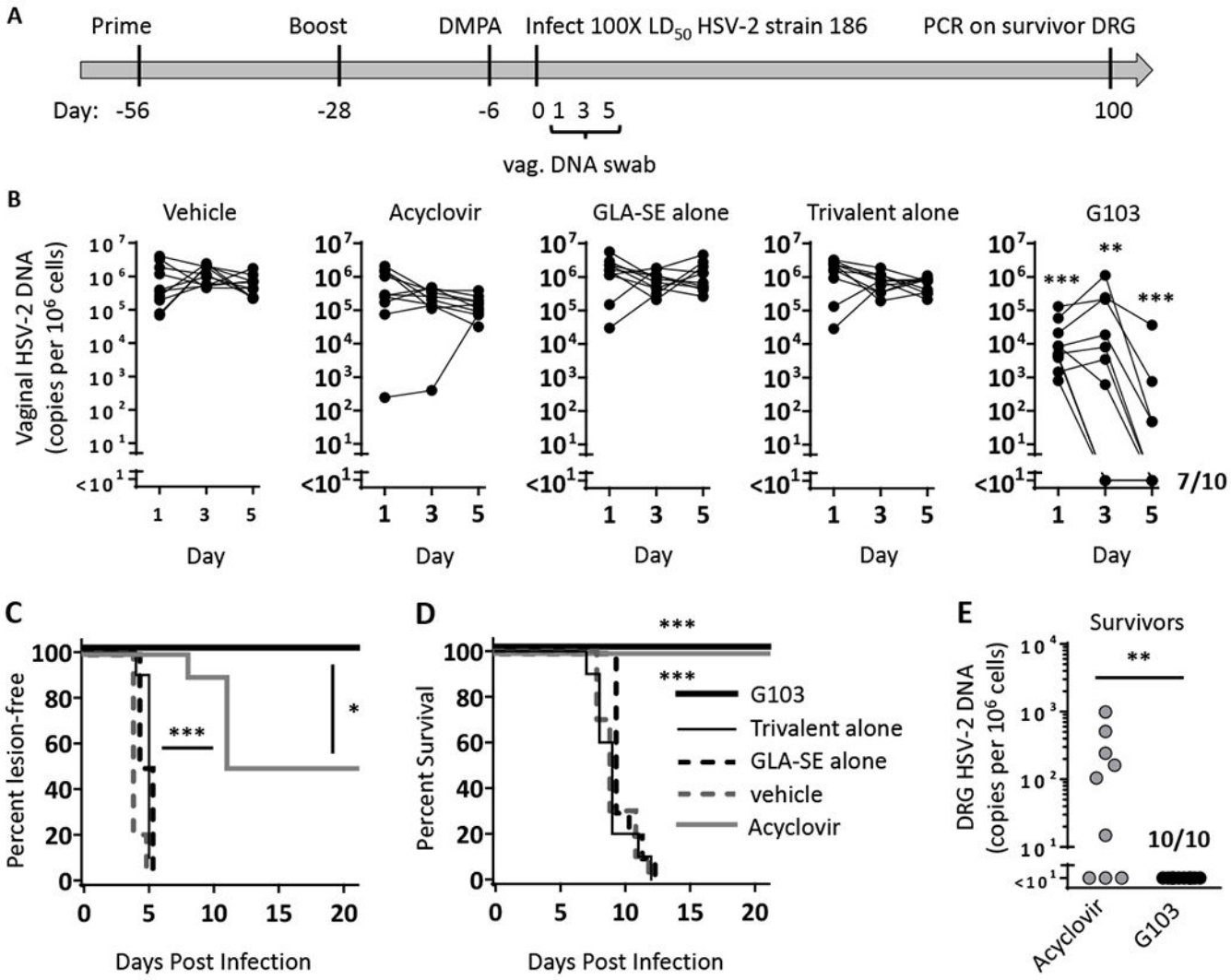

Figure 2. Prophylactic immunization leads to complete survival after lethal challenge, protection from genital lesions, and prevention of latent infection.

A) A schematic of the challenge experiment. Mice were immunized twice 28 days apart with G103 or the unadjuvanted trivalent vaccine. Negative control vaccines included GLA-SE adjuvant alone and vehicle (5% dextrose) alone. 4 weeks post boost, mice were challenged with a 100x LD50 dose of HSV-2 strain 186 following pretreatment with depot medroxyprogesterone acetate (DMPA; Depo-Provera). As a control group of surviving mice with latent infection, one group of unimmunized mice were treated orally with acyclovir from days 1-21. B) Vaginal swabs were collected on days 1, 3, and 5 post infection and copies of HSV-2 copies were measured by quantitative PCR. For each time point, statistically significant differences compared to the pooled other vaccine groups are indicated by ** ($p<0.01$) or *** ($p<0.0001$) (Mann-Whitney test). Kaplan-Meier curves showing the percentage of mice that were C) free of genital lesions and D) surviving up to 21 days post infection is shown. Significance indicated by * ($p<0.05$) or *** ($p<0.0001$) (Logrank test). Note, one acyclovir treatment mouse died after day 21. E) On day 100, the

dorsal root ganglia (DRG) were dissected from surviving mice and the copy number of latent HSV-2 was measured by qPCR. **p<0.01 (Mann-Whitney test).

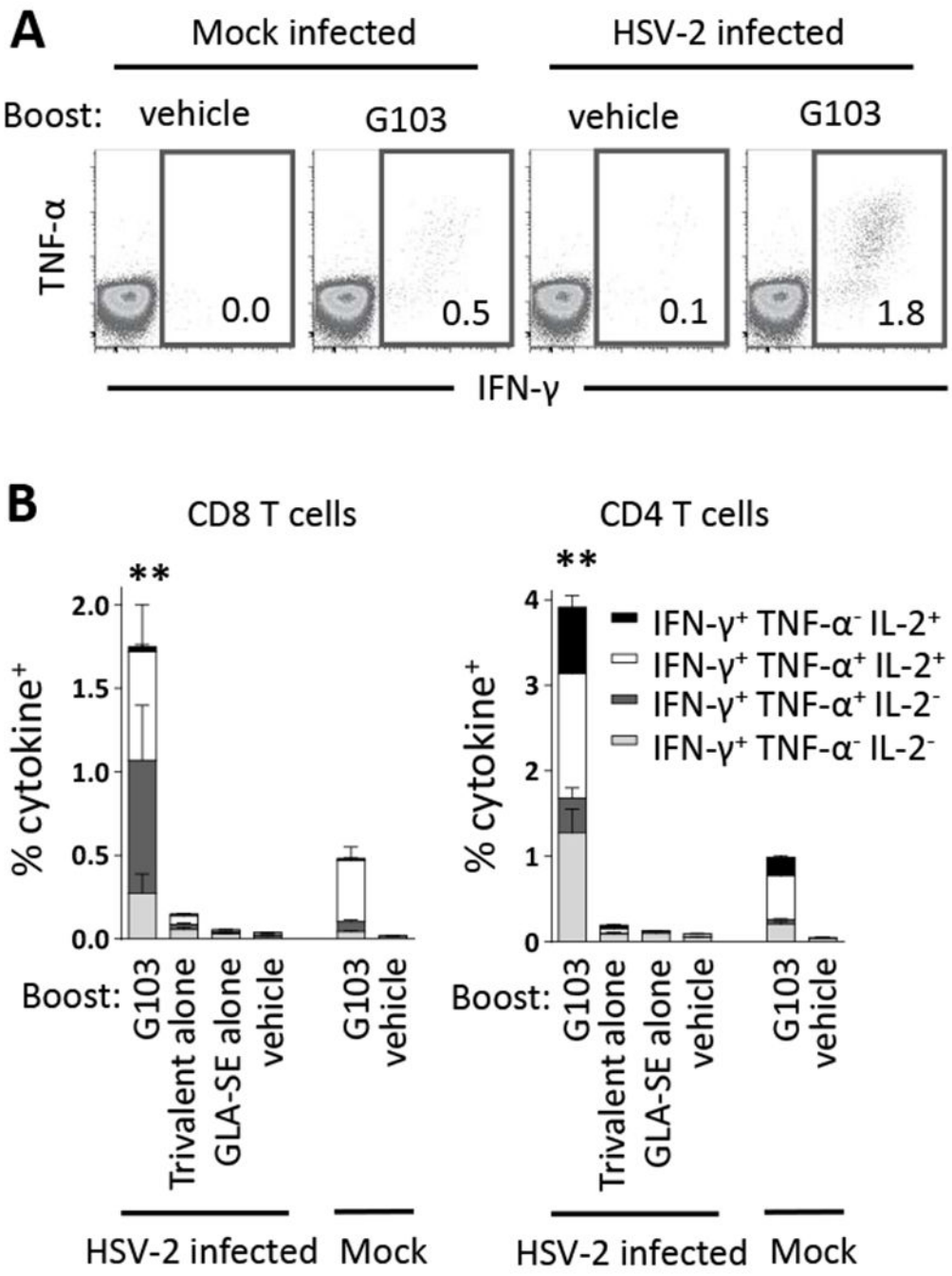

Figure 3. G103 expands virus-specific memory CD4 and CD8 T cells in mice previously infected with HSV-2.

Mice (n = 5 per group) were infected subcutaneously with HSV-2 Tk$^-$ virus or mock and were then immunized 28 days later with G103 or the indicated controls. 6 days post immunization, the frequency of UL19-specific T cells was measured by ex vivo peptide pool restimulation followed by ICS. A) Representative plots of UL19-specific CD8 T cell response. B) Bar graphs showing the frequency and multifunctionality of UL19-specific CD8 T cells and CD4 T cell responses. Statistically significant differences in the memory response to G103 is indicated by ** ($p<0.01$) (Mann-Whitney test).

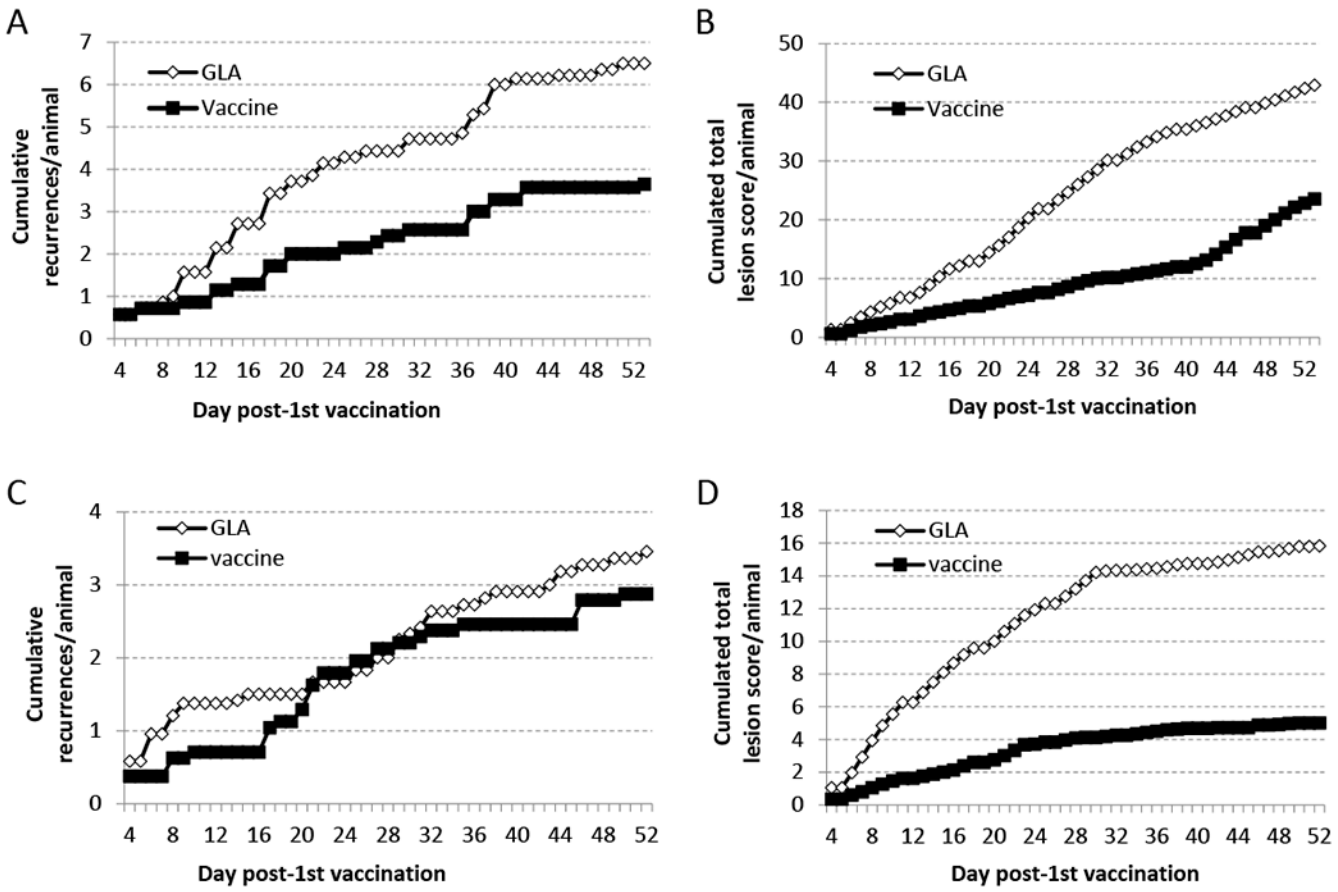

Figure 4. Therapeutic vaccination reduces the number of recurrent genital lesions in HSV-2 infected guinea pigs.

Guinea pigs were infected twice intravaginally with HSV-2 strain 333. On days 5, 7, 9, and 11 after the second infection, genital lesions were graded on a scale of 0 for no lesions, 1 for erythema, 2 for single or a few small vesicles, 3 for large or fused vesicles, and 4 for ulcerated lesions. Guinea pigs were then separated into two groups matched for lesion score and randomly assigned to either treatment with trivalent vaccine with GLA-SE adjuvant or adjuvant alone. Treatments were administered intramuscularly on days 14 and 35 post-infection. Two separate experiments (A and B, C and D) were performed. Starting on the fourth day after the first treatment, recurrent genital lesions were counted and scored as above, and the cumulative frequency (A, C) and severity (B, D) of lesions in each group is shown.