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Prospects and Perspectives for Development of a Vaccine Against Herpes Simplex Virus Infections

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Abstract

Herpes simplex viruses 1 and -2 are human pathogens that lead to significant morbidity and mortality in certain clinical settings. The development of effective antiviral medications, however, has had little discernible impact on the epidemiology of these pathogens, largely because the majority of infections are clinically silent. Decades of work have gone into various candidate HSV vaccines, but to date none has demonstrated sufficient efficacy to warrant licensure. This review examines developments in HSV immunology and vaccine development published since 2010, and assesses the prospects for improved immunization strategies that may result in an effective, licensed vaccine in the near future.

Keywords

Herpes simplex; vaccination; latency; glycoproteins; clinical trials; cell mediated immunity; humoral immunity

Introduction

Herpesviruses all share a general virion structure that includes a large, linear, double-stranded DNA genome that is densely packed within an icosahedral capsid [1]. The capsid is surrounded by an amorphous tegument that is made up of more than 30 viral proteins. Finally, the virion is surrounded by a lipid envelope that is studded with multiple viral glycoproteins as well as some cellular proteins.

The eight known human Herpesviruses are divided into three subfamilies: *Alphaherpesvirinae* (Herpes simplex-1 and -2, Varicella-zoster virus); *Betaherpesvirinae* (Cytomegalovirus, Human Herpesvirus-6 and -7); and *Gammapherpesvirinae* (Epstein-Barr virus, Kaposi sarcoma Herpesvirus). Members of the three subfamilies differ in genomic content, routes of transmission, sites of latency, clinical manifestations of viral reactivation, and susceptibility to current anti-viral medications [1].

Herpes simplex virus- (HSV-) 1 and 2 have considerable medical impact across all geographic and socioeconomic divisions. HSV infections are common and ubiquitous. Approximately two-thirds of the world's population is infected with herpes simplex virus

type 1 and/or type 2 (HSV-1 & HSV-2) that cause ocular, oro-facial (cold sores) and genital herpetic diseases [2]. Primary infection with HSV-1 & HSV-2 in immunocompetent individuals is usually asymptomatic and results lifelong viral latency in sensory ganglia and both the ubiquitous nature of infection and well as the propensity for the virus to cause latency are complicating variables in vaccine development [3,4]. HSV also encodes a plethora of immune evasion genes that complicate the concept of vaccine development [5-7]. HSV-1 is classically associated with oculo-facial infections and HSV-2 with genitourinary infections. However, this anatomical distinction is no longer considered to be as strict [8]. Ocular infections with HSV-1 are the leading cause of infectious blindness in the developed world, with recurrent episodes of viral reactivation leading to progressive scarring and opacity of the cornea [9]. HSV-2 is sexually transmitted and estimated to infect 500 million people worldwide including 23 million new infections per year [10]. Seroprevalence is 16% among 14 to 49 year olds in the United States, with rates in women nearly twice that of men [11,12]. Seroprevalence tends to be higher in developing countries with rates of 80% found in some Sub-Saharan African nations [13].

Acquisition of HSV by infants during parturition can result in serious morbidity and mortality [14-16]. Mothers who acquire infection during pregnancy are at highest risk of transmitting HSV to infants because of higher titers of virus in vaginal fluid and lack of transplacental transfer of HSV-specific IgG to the infant [17]. Numerous studies have evaluated screening protocols for pregnant women, anti-viral prophylaxis of HSV-positive pregnant women, and caesarian delivery in preventing transmission to neonates [17-21]. None of these measures, however, has shown significant protection.

Of grave concern is the fact that genital infection with HSV-2 increases the risk of acquiring HIV infection by three-fold [22]. In countries with high HSV-2 seroprevalence, as much as 50% of HIV infections are attributable to antecedent genital herpes [23]. This increased risk is not due to disruption of the genital mucosa by reactivation as asymptomatic HSV-2 infections have the same rate of transmission and HSV-2 suppression with anti-viral medications does not mitigate risk [24-26]. Latent HSV-2 infection is associated with a robust influx of antigen-specific CD4⁺ T cells, thus increasing the local concentration of cells permissive for HIV infection [27-29].

One reason an effective vaccine is urgently needed is that asymptomatic shedding of transmissible virus occurs in the absence of clinical symptoms in the majority of cases [30,31]. In fact, following primary infection the majority of infected individuals never has clinically evident reactivations, and therein lies the failure of antiviral medications to dramatically alter the epidemiology of HSV infection [12]. Serological studies have established that only 10% to 25% of seropositive subjects in the United States is aware that they are infected [11,12,31]. Recent studies that have employed extensive ano-genital sampling of infected, asymptomatic individuals have demonstrated that clinically-silent viral shedding occurs frequently and in short bursts [29,32-34]. Thus, anti-viral medication prescribed only to individuals with clinically apparent reactivations has no bearing on transmission resulting from asymptomatic viral shedding. The finding that the majority of new infections passed within sero-discordant couples in the absence of clinical disease underscores the importance of silent reactivation. Furthermore, the reliance of a history of

genital herpes lesions in pregnant women is insufficient to accurately determine the risk of perinatal transmission of HSV infection [35]. The challenges of vaccination of seropositive individuals are formidable and beyond the scope of this review, although they have been reviewed in several excellent papers [4,36]. For purposes of this analysis, HSV-seronegative women of child-bearing age are considered the major target population for vaccine development, given the devastating consequences of neonatal HSV infection (reviewed below) [37,38]. Vaccine development targeting at decreasing asymptomatic viral shedding, or limiting recurrent infection rather than preventing primary infection, may be an appropriate endpoint in clinical trial design for some populations.

Primary Infection

Primary infection of multiple cell types, including epithelial cells, fibroblasts, lymphocytes, and neurons, with HSV-1 and -2 proceeds by in a stepwise fashion beginning with the interaction between infectious viral particles and the glycocalyx of permissive cells [39]. The subsequent fusion of the viral envelope with a host cell lipid membrane then delivers the viral capsid to the cytoplasm. Unlike other enveloped viruses such as vesicular stomatitis virus and varicella zoster virus, where attachment and fusion events are mediated by a single viral glycoprotein, the HSVs encode for at least a dozen glycoproteins that mediate viral entry [39]. Some of these glycoproteins are essential for infection while others increase the efficiency of infection but are not necessary.

The classic view of HSV entry is that viral particles fused directly with the host cell plasma membrane at neutral pH. However, electron micrographs of infected cells have demonstrated the presence of enveloped viral particles within the host cell cytoplasm [40]. Subsequent work has demonstrated that HSV infection can be mediated by the endocytic pathway, in a pH-dependent or -independent manner [41-43]. Both the classic and endocytic entry pathways require the same four glycoproteins: gB, gH/gL, and gD [44,45]. Subtle differences in these pathways, however, may in part explain the failure of glycoprotein-based subunit vaccine candidates (discussed below).

Irrespective of the route of entry, viral particles must de-envelope in order to release the viral capsid into the host cell cytosol. De-enveloped capsids are then transported to the nuclear membrane along microtubules [46] and interact with nuclear pore complexes leading to extrusion of viral DNA into the nucleus [47]. Upon delivery of the viral genome to the nucleus two types of infection may proceed: latent or lytic.

Latent and Lytic Replication

A distinguishing feature of Herpesviruses is the establishment of latency following primary infection [48]. Latency is typified by expression of few or no viral proteins and RNA transcripts, and maintenance of the viral genome as circularized episome that is maintained in the host cell nucleus by tethering to histone proteins. Following initial productive infection primarily in epithelial cells, retrograde axonal transport delivers viral DNA to sensory neurons within dorsal root ganglia, the site of latency for all the alpha Herpesviruses [49]. Oculo-facial infections resolve with establishment of latency in the

trigeminal ganglia, whereas genital infections lead to latency in the pelvic splanchnic neurons.

Periodically lytic replication may be induced. Extensive remodeling of the nucleus accompanies productive viral infection [50,51]. Expression of viral genes proceeds in a coordinated cascade beginning with immediate early genes, the expression of which does not require translation of any viral proteins [52]. Delayed early genes follow prior to the onset of viral DNA replication. Both immediate and delayed early genes have regulatory functions. Following viral DNA replication, expression of late genes, which code for predominantly structural proteins, allows for packaging of viral DNA copies into new capsids. Acquisition of tegument proteins and a host cell-derived lipid envelop studded with viral glycoproteins results in release of infectious viral progeny [53].

Innate and Adaptive Immunity to HSV

The ability to induce expression of neutralizing antibodies has been the gold standard of assessing vaccine efficacy in pre-clinical and clinical trials. However, the role of humoral immunity in preventing primary infection with HSV and limiting the extent of reactivation is less clear. For example, primary immunodeficiencies typified by reduced humoral immunity, such as common variable immunodeficiency or agammaglobulinemia, generally are not associated with increased risk of severe viral infections [54]. One reason humoral responses do not fully eliminate viral shedding is that many infectious virions produced during lytic replication spread laterally at the junctions between epithelial cells, rather than at apical membranes where antibody would have access to viral particles. Lateral spread of viral particles is mediated by viral glycoproteins gE and gI, but the inability of antibodies to gain access to the paracellular compartment makes these glycoproteins poor vaccine candidate antigens [55,56]. Neutralizing antibodies undoubtedly have a role, however, in mitigating the risk of perinatal transmission. Transplacental transfer of IgG specific for HSV to the developing fetus likely contributes to the reduced risk of neonatal infection in infants born to mothers whose primary HSV infection preceded pregnancy [17].

The essential role of cell mediated immunity (CMI) in control of HSV infection can be inferred from the fact that reduced CMI responses due to iatrogenic immune suppression or HIV infection lead to a significantly increased risk of developing severe HSV infections [57,58]. Recruitment of antigen-specific CD8⁺ T cells to lesional tissue is a necessary step in clearing infectious virus and infected cells [59]. Biopsy specimens from genital infections have furthermore demonstrated an inverse correlation between lesional CD8⁺ T cell content and the severity of viral reactivation [29]. Recruitment of CD4⁺ T cells is also essential for controlling infection; however, the influx of cells that are susceptible to infection with HIV has been proposed as one mechanism by which genital HSV infections increase the risk of HIV transmission [27-29]. A detailed discussion of the range of epitopes recognized in the context of HSV infection is beyond the scope of this review, which is intended to provide an update of vaccines currently in the clinic, but the immunology of the anti-HSV response has been the subject of several recent papers [36,60,61]. Of particular note is the importance of CD8⁺ T cell epitopes gB342-350 and gB561-569 in protection against reactivation, both in seropositive asymptomatic individuals and in protection of HLA transgenic mice against

ocular HSV infection [62]. Another elegant recent study, employing cell-type-specific laser capture microdissection, transcriptional profiling and T-cell antigen receptor beta-chain (TCR-beta) genotyping on sequential genital skin biopsies, showed that CD8alphaalpha(+) T cells are the dominant resident population of DEJ CD8(+) T cells that persist at the site of previous HSV-2 reactivation [63]. Hopefully, elicitation of these key cells for immune surveillance and initial containment of HSV-2 reactivation in human peripheral tissue can be the basis for future innovative vaccine approaches.

In addition to adaptive immune responses, recent evidence implicates the innate immune system in protection from severe HSV infection. The intracellular toll like receptors (TLR) 3 and 9 have been shown to be necessary for induction of interferon responses to HSV infection. Interferons are essential for inducing an anti-viral state in neighboring cells, inducing recruitment of CD8+ T cells to sites of HSV infection, and stimulating maturation of dendritic cells, which are indispensable in priming adaptive CTL responses. Likewise, natural killer cell recruitment and activation is in part dependent on interferon signaling. Augmentation of immune responses to HSV has been achieved in murine models with co-administration of the TLR9 agonist cPG [64]. Adjuvant development focused on maximizing stimulation of innate immunity may have a large impact on future candidate HSV vaccines [65,66].

Lessons from Animal Models of HSV Vaccines

Animal models for HSV infection have been important in furthering our understanding of key pathophysiological mechanisms [67]. However, there are important differences in the natural history of HSV infection in humans versus mice. Most importantly, primary HSV infection of mice does not lead to a persistent, predominantly latent infection as it does in humans. Accordingly, murine studies rely on lethality as an endpoint, which is not necessarily relevant to the natural history of HSV infection in humans. Guinea pigs, in contrast, do establish latency and undergo spontaneous reactivation in the genital track. Though more physiologically relevant to humans, the guinea pig model suffers from a paucity of reagents making it more difficult to conduct in depth immunological analyses. The use of mouse and guinea pig models for studying interventions against genital HSV infection is the subject of several recent reviews [67,68]. Some of the more instructive preclinical vaccine studies from these animal models are reviewed below.

Some of the first animal model studies of HSV vaccines were performed in the 1970s. Experimental evaluation of vaccines for genital HSV infection has largely been performed in the guinea pig model, because of its unique relevance to human HSV-2 disease - in particular, the propensity for animals infected by vaginal route to undergo spontaneous genital tract recurrences [69-71]. Early studies mostly consisted of evaluation of immunogenicity of crude glycoprotein extracts, or evaluation of immune response following animal inoculation with virus [72-76]. The first suggestion that these proteins might be effective in controlling acute and recurrent genital HSV disease came in 1981, when Thornton et al. reported that vesicles of plasma membrane prepared by treatment of HSV2-infected Vero cells with dithiothreitol and formaldehyde were antigenic in guinea-pigs and also protected these animals against intravaginal challenge with HSV-2 [69]. The advent of cloning technologies

for expression of viral glycoproteins enabled investigation of adjuvanted, recombinant HSV-1 gD as a candidate vaccine in the guinea pig model. Studies reported by Berman et al. indicated that vaccinated animals formed antibodies that neutralized both HSV-1 and HSV-2, and were protected against disease (although not against recurrent infection) following challenge with HSV-2 by intravaginal route [77,78]. Studies using lectin-purified gD and gB subunit vaccine demonstrated similar protection, not only against primary infection, but recurrent disease [79]. Adjuvant choice plays a crucial role in protection in adjuvanted glycoprotein studies in the guinea pig model, with adjuvants capable of eliciting robust cellular immune responses in addition to antibody responses appearing to enhance protection against disease [64,80-86]. Other novel routes of delivery of adjuvanted HSV glycoproteins have been evaluated, including intranasal administration [87,88]. Variations of this approach have included recombinant adjuvanted HSV gD vaccine co-administered with the ICP4 protein [89], or in combination with another HSV glycoprotein, gC [90]. Another interesting study examined the proprietary adjuvant IC31 in combination with HSV-2 glycoprotein gD administered via intranasal, intradermal, or subcutaneous routes. Immunization conferred 80-100% protection against an otherwise lethal vaginal HSV-2 challenge with amelioration of viral replication and disease severity in the vagina [91]. Adjuvanted combinations of three glycoproteins, gB, gD and gE, have been evaluated in the guinea pig model, using aluminum hydroxide-based adjuvants [92]. In this study, guinea pigs were vaccinated after establishment of recurrent genital HSV disease. Immunization results in a reduction in recurrences, supporting a potential role for an HSV vaccine as an immunotherapeutic approach for individuals with recurrent genital HSV infection.

In addition to recombinant protein vaccines, a number of preclinical studies have been performed in the guinea pig genital HSV model with DNA vaccines. Initial studies focused on gD and gB plasmid vaccines [93-96], either alone, in combination, or in a “prime-boost” sequence of DNA vaccine priming followed by recombinant protein boosting [97]. As with recombinant glycoprotein, addition of adjuvant to HSV DNA vaccines appears to be important in maximizing immune response. One study demonstrated efficacy of a gD-based DNA vaccine when administered with bupivacaine [98]. A DNA-based vaccine developed by Vical corporation that includes the coding region for full-length gD2 plus a proprietary cationic lipid-based adjuvant, Vaxfectin, demonstrated excellent protection in a murine model of lethal intravaginal challenge [99]. Compared with unadjuvanted immunized animals, mice immunized with full the full length gD2-encoding plasmid in conjunction with Vaxfectin had significantly improved survival at 20 days following HSV challenge with 500 times the LD₅₀ (0% v. 80%, unadjuvanted v. adjuvanted, respectively). The only correlate of protection besides survival tested was antibody production. Vaxfectin-adjuvanted HSV-2 DNA vaccines have also been studied in the guinea pig model of genital HSV disease [100]. This study examined a gD2 plasmid vaccine combined with HSV-2 UL46 and UL47. Vaccination significantly reduced viral replication in the genital tract, provided complete protection against primary and recurrent genital skin disease following intravaginal HSV-2 challenge, and significantly reduced latent HSV-2 DNA in the dorsal root ganglia compared to controls. The vaccine also reduced the frequency of recurrences in animals already latently infected. Another recent DNA vaccine study in a mouse HSV-2 challenge model indicated that the addition of ICP27 to gD- and gB-based vaccines

regimens improved efficacy, presumably by an enhancement of the T cell response [101]. Finally, HSV-2 gD2 DNA vaccines were recently examined in combination with two conserved HSV-2 genes necessary for virus replication, UL5 (DNA helicase) and UL30 (DNA polymerase), in a “prime-boost” approach with formalin-inactivated HSV-2 (FI-HSV2) formulated with monophosphoryl lipid A (MPL) and alum adjuvants, in a mouse model of genital HSV-2 challenge [102]. Overall, DNA HSV-2 vaccines continue to be an area of intensely active research, and human trials are warranted. Indeed, two DNA vaccine plasmid constructs encoding HSV-2 gD have moved forward in phase 1 clinical trials [103], and are considered in the next section of this review.

Other recent pre-clinical developments in live-attenuated HSV vaccine methods deserve mention. An ICP0 “knock-out” virus showed significantly greater protection in lethal dose experiments in mice compared with a subunit vaccine preparation of gD2 [104]. Viral shedding was also significantly reduced following live-attenuated vaccine strain administration over subunit vaccination. The only immune parameter assessed in initial studies was production of anti-gD neutralizing antibodies, although a follow up study measured the pan-HSV-2 antibody response following vaccination with ICP0 deletion vaccines, and found in mice and guinea pigs quantitatively correlation between both reductions in viral shedding and increased survival frequency following HSV-2 challenge [105]. Further analyses of novel correlates of protective antibody responses, as well as the induction of CMI in this model, would be of great interest and may help move this interesting candidate vaccine forward into clinical testing.

Another interesting and novel vaccine strategy dubbed “prime and pull” was recently published [106]. Vaccination of mice with an attenuated HSV-2 strain followed by a single topical application of the T cell-tropic chemokines CXCL9 and CXCL10 to the vaginal mucosa of vaccinated mice resulted in significant recruitment and retention of virus-specific effector memory T cells. This recruitment resulted in significantly improved survival in subsequent lethal challenge studies. Though in the present two-step form this strategy may be difficult to implement in human trials, the underlying validation of the prime importance of local CMI in HSV immunity is important. Less invasive methods of improving recruitment of effector memory T cells to mucosal compartments may be essential to developing HSV vaccines effective in humans.

Recent and Ongoing Clinical Trials of HSV Vaccines

A summary of ongoing clinical trials is provided in Table 1. The viral glycoproteins gD and gB elicit the strongest neutralizing antibody titers to HSV-2 and have demonstrated varying degrees of efficacy in animal models, as reviewed above, and have therefore received the most attention from vaccine developers. However, the results with glycoprotein subunit vaccines have been generally disappointing. Recently, a subunit glycoprotein vaccine by GlaxoSmithKline failed to reduce acquisition of primary HSV-2 infection in seronegative women of reproductive age [107,108]. The recombinant truncated gD2 vaccine administered with alum and 3-*O*-deacylated monophosphoryl lipid A adjuvants initially showed promise in preventing HSV-2 transmission to women but not men within sero-discordant couples [109]. However, in the follow up trial that enrolled over 8000 HSV-1 and HSV-2 uninfected

women ages 18 to 30, no benefit was found in preventing acquisition of HSV-2 infection after up to three doses of vaccine despite the ability of the vaccine to generate neutralizing antibody. Furthermore, the vaccine did not reduce the level of viral shedding in those women who acquired HSV-2 infection during the study period. Surprisingly, there was a small but significant reduction in acquisition of HSV-1, despite the inclusion of only the HSV-2 gD protein in the vaccine. The ~80% sequence identity between the gD proteins of HSV-1 and -2 may have led to cross reactivity. It is also known that antecedent HSV-2 infection is partially protective against subsequent HSV-1 infection, though not vice versa REF.

A subunit vaccine by Genocera, GEN-003, that contains truncated recombinant gD2 and ICP4 has shown promise in preclinical studies [89]. Using a novel and proprietary screening method to identify immune-dominant epitopes, Genocera demonstrated that CD8+ clones reactive to ICP4 distinguished asymptomatic HSV-2-positive or HSV-2-exposed but seronegative individuals from HSV-2-positive patients with frequent, clinically-evident reactivations. The bivalent vaccine adjuvanted with a proprietary, saponin-based compound Matrix M™ stimulated production of neutralizing antibody in immunized mice, as well as CD4+ and CD8+ responses that demonstrated anamnestic responses up to 44 days after vaccination. Vaccination of HSV-2-infected guinea pigs, which develop a clinically-relevant latent/lytic chronic phase of infection, reduced the incidence and severity of reactivation as well as reducing the level of asymptomatic shedding [89]. These findings suggested that the Genocera vaccine is a potentially efficacious therapeutic vaccine candidate. Accordingly, a phase 1/2a double-blind, placebo-controlled, dose-escalation trial is now enrolling with a goal of recruiting approximately 150 subjects with moderate to severe HSV-2 infection ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01667341) identifier NCT01667341). The primary endpoints of this trial are safety, tolerability, and reduced viral shedding.

Promising results were published following a phase I trial of another novel HSV-2 vaccine formulation, HerpV, manufactured by Aenus [110]. Recombinant human heat shock protein 70 (rhHsc70) non-covalently linked to synthetic HSV-2 peptides adjuvanted with the proprietary saponin-based compound QS-21 (Stimulon®) was administered to 35 HSV-2-infected subjects. Notably, the vaccine includes 32 peptides derived from 22 viral proteins that cover all phases of the viral life cycle. The high valency of the study vaccine induced broad CD4+ and CD8+ responses as measured by cell proliferation assays and ELISPOT. Of concern, transient antibody responses to HSC70 were detectable after up to three doses of the study vaccine, though no evaluation of CMI to HSC70 was reported. The potential for eliciting an auto-immune response needs to be closely evaluated as this otherwise promising vaccine candidate proceeds through clinical testing. Based on these promising results, a phase II double-blind, placebo-controlled trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01687595) identifier NCT01687595) is now enrolling with a recruitment goal of 75 adults with recurrent HSV-2 infection. The primary endpoint of this trial is reduced viral shedding during the treatment period versus baseline period.

A phase I study of a live, replication-defective HSV-2 vaccine, HSV529, is planned by Sanofi Pasteur ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01915212) identifier NCT01915212). This virus, dl5-29, fails to form plaques or to give any detectable single cycle yields in normal monkey or human cells,

but expresses nearly the same pattern of gene products as wild-type virus, and induces antibody titers in mice that are equivalent to those induced by single deletion mutant viruses [111]. Further improvements of immunogenicity for this virus have been reported in mice after deletion of the UL41 gene [112], although the triple deletion virus did not appear to provide better protection against HSV-2 challenge in mouse or guinea pig studies [113,114]. In addition, a comparison of this vaccine to adjuvanted gD2 demonstrated its superiority in the guinea pig model of genital HSV-2 infection [115]. A phase I study of the double-deletion virus is currently underway to (a) determine the safety of HSV529, in persons with or without HSV infection; and (b) examine the ability of HSV529 to elicit immune responses to HSV-2 including virus-specific antibodies and T cell responses. The approach has similarities to that of disabled, infectious, single-cycle (DISC) vaccines developed against HSV-1 and HSV-2 by Cantab Pharmaceuticals in the 1990s. These DISC vaccines were based on deletion of the gH gene, which was then supplied by a complementing cell line: hence, the virus could bind, attach, and initiate gene expression, but only undergo a single cycle infection. Although these vaccine showed promise in preclinical models of HSV infection [116-118], when studied in a clinical trial in patients with recurrent genital infection, no clinical or virological benefit in the amelioration of genital HSV-2 disease could be identified [119], and the vaccine did not undergo any further clinical trial testing.

Expert Commentary

The recent high-profile failure of the GlaxoSmithKline vaccine to prevent transmission of HSV infection is likely multifactorial, involving the inherent challenges posed by a highly adapted pathogen (discussed below) as well as issues related to trial design. The initial promising findings with the GlaxoSmithKline vaccine may have been the result of the stringent selection criteria for subject enrollment. Sero-discordant, monogamous couples may not accurately represent the immunological characteristics of the wider population that acquire a primary HSV infection. Others have shown that up to a third of uninfected partners in sero-discordant couples have HSV-specific cell mediated immunity despite lacking evidence of an antibody response [120]. Such “immune-seronegative” subjects that remain uninfected despite repeated exposures from an infected partner may not be immunologically similar in heretofore unknown ways; findings in such subjects may therefore have limited generalizability.

The *in vitro* neutralizing activity of antibodies induced by experimental vaccines may not reflect actual neutralizing ability *in vivo*. Notably, plaque reduction assays used in laboratory analysis to detect neutralizing antibodies employ cell lines, usually of epithelial origin, that may bear little or no resemblance to the permissive cell types infected *in vivo*. As mentioned, there are many permissive cell types involved in primary infection, each with potentially different glycoprotein requirements. Furthermore, plaque assays are performed at neutral pH but normal vaginal pH is significantly acidic (pH 3.5 – 4.5). Though viral glycoproteins are thought to function during viral entry via the classic pathway irrespective of pH, the endocytic pathway has been found to be pH dependent in certain cell types [41,42]. Subtle differences in conformation induced by acidic pH have been demonstrated in gB [121,122], a viral antigen included in numerous subunit vaccines. These differences may be important in escaping neutralization in some settings but not others such that despite

blockade of the classic entry pathway infection can still be established by the alternative endocytic pathway. Additionally, it is unclear if “neutralizing” concentrations of HSV-specific IgG accumulate at mucosal sites following intramuscular injection of vaccine candidates.

An important challenge for vaccine development is that assays to evaluate CMI following experimental vaccine challenge are expensive, cumbersome, and not widely standardized or agreed upon. Assessment of a proliferative response to viral antigen, for example, gives no insight into functionality of the activated clones. However, while assessment of functionality (production of cytokines, granzyme, perforin, etc.) provides insight into the robustness of the antigen, the lack of validated correlates of CMI protection makes using such data to guide vaccine development difficult.

Choice of antigens for inclusion in future vaccine candidates should be informed by new immunological data. As mentioned, up to a third of seronegative partners of HSV-positive subjects are immune-seronegative with robust adaptive cell mediated immune responses to HSV antigens. In the largest study of immune-seronegative subjects published to date, the most common antigen-specific responses were to ICP6, ICP4, ICP0, UL19, and UL29 [120]. The skewing of reactivity towards immediate early proteins was in sharp contrast to the CMI responses of the sero-positive partners where late genes, including gD and gB, were more common. Another recent study evaluated the humoral responses to HSV in ten asymptomatic (defined as up to one reactivation per year) and ten symptomatic subjects (defined one to five reactivations per year) [123]. Reactivity to gD and gB did not distinguish these groups but similar to the immune-seronegative reactivity, the asymptomatic group was significantly more likely to have responses to early viral proteins (in this case UL30 and UL42, two proteins necessary for viral DNA replication. Another novel approaches to discovery of new T cell epitopes should provide insight into the components of HSV-2 vaccines that are likely to perform successfully in the clinic [63,124-126].

To date the only successful vaccine developed for a human Herpesvirus infection is the live attenuated *Oka* strain varicella-zoster vaccine [127]. Several differences between VZV and HSV are likely to contribute to this, including sustained viremia following primary VZV but not HSV infection [128], allowing prolonged access by neutralizing antibody prior to establishment of latency; expression of multiple latency genes by VZV but none by HSV, [128], thus providing ongoing stimulation of the adaptive arms of the immune system following primary VZV; and the relative paucity of immune evasion strategies employed by VZV, which is in sharp contrast to the multiple known immune evasion strategies of HSV [129,130]. Lastly, given the near constant shedding of HSV but not VZV by asymptomatic individuals it has been proposed that natural immunity to HSV is not as robust as that for VZV [130]. In the face of a more well-adapted pathogen, we may be attempting to exploit immune mechanisms that are inherently insufficient.

Five-Year Commentary

Development of glycoprotein subunit vaccines needs to take into account possible pH-dependent conformational epitopes. Additionally, optimization of epitope inclusion into future candidate vaccines will be necessary. Clues from clinical observations that point to the importance of early antigens in protecting against transmission between sero-discordant couples and that also distinguish asymptomatic from symptomatic HSV carriers should guide antigen selection.

Further understanding of aspects of CTL functionality will help define and validate correlates of protection. The evidence supporting the need to elicit robust CTL responses is overwhelming, but our tools to design a perfect vaccine able to do so is limited. Improvements in eliciting therapeutic CTL responses may be aided by advances in adjuvant science.

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References

* Of interest

** Of considerable interest

1. Knipe, DM.; Howley, PM.; Griffin, DE. Fundamental virology. Lippincott Williams & Wilkins; Philadelphia: 2001.
- 2*. Dasgupta G, Chentoufi AA, Nesburn AB, Wechsler SL, BenMohamed L. New concepts in herpes simplex virus vaccine development: notes from the battlefield. *Expert Rev Vaccine*. 2009; 8(8): 1023–1035. Excellent overview of key issues facing HSV vaccine development, including critical roles of evaluating T cell response antigens and counteracting viral immune evasion.
3. Chentoufi AA, BenMohamed L, Van De Perre P, Ashkar AA. Immunity to ocular and genital herpes simplex viruses infections. *Clin Dev Immunol*. 2012; 2012:732546. [PubMed: 23326289]
4. Chentoufi AA, Benmohamed L. Mucosal herpes immunity and immunopathology to ocular and genital herpes simplex virus infections. *Clin Dev Immunol*. 2012; 2012:149135. [PubMed: 23320014]
5. Coleman JL, Shukla D. Recent advances in vaccine development for herpes simplex virus types I and II. *Hum Vaccin Immunother*. 2013; 9(4):729–735. [PubMed: 23442925]
6. Awasthi S, Huang J, Shaw C, Friedman HM. Blocking HSV-2 glycoprotein E immune evasion as an approach to enhance efficacy of a trivalent subunit antigen vaccine for genital herpes. *J Virol*. 2014
7. Ndjamien B, Farley AH, Lee T, Fraser SE, Bjorkman PJ. The herpes virus Fc receptor gE-gI mediates antibody bipolar bridging to clear viral antigens from the cell surface. *PLoS Pathog*. 2014; 10(3):e1003961. [PubMed: 24604090]
8. Ryder N, Jin F, McNulty AM, Grulich AE, Donovan B. Increasing role of herpes simplex virus type 1 in first-episode anogenital herpes in heterosexual women and younger men who have sex with men, 1992–2006. *Sex Transm Infect*. 2009; 85(6):416–419. [PubMed: 19273479]
9. Farooq AV, Shukla D. Herpes simplex epithelial and stromal keratitis: an epidemiologic update. *Surv Ophthalmol*. 2012; 57(5):448–462. [PubMed: 22542912]
10. Looker KJ, Garnett GP. A systematic review of the epidemiology and interaction of herpes simplex virus types 1 and 2. *Sex Transm Infect*. 2005; 81(2):103–107. [PubMed: 15800084]
11. Seroprevalence of herpes simplex virus type 2 among persons aged 14–49 years--United States, 2005–2008. *MMWR Morb Mortal Wkly Rep*. 2010; 59(15):456–459. [PubMed: 20414188]

12. Xu F, Sternberg MR, Kottiri BJ, et al. Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. *JAMA*. 2006; 296(8):964–973. [PubMed: 16926356]
13. Paz-Bailey G, Ramaswamy M, Hawkes SJ, Geretti AM. Herpes simplex virus type 2: epidemiology and management options in developing countries. *Postgrad Med J*. 2008; 84(992): 299–306. [PubMed: 18644920]
14. Kimberlin D. Herpes simplex virus, meningitis and encephalitis in neonates. *Herpes : the journal of the IHMF*. 2004; 11(Suppl 2):65A–76A.
15. Kimberlin DW, Lin CY, Jacobs RF, et al. Natural history of neonatal herpes simplex virus infections in the acyclovir era. *Pediatrics*. 2001; 108(2):223–229. [PubMed: 11483781]
16. Long SS, Pool TE, Vozzak J, Daskalaki I, Gould JM. Herpes simplex virus infection in young infants during 2 decades of empiric acyclovir therapy. *Pediatr Infect Dis J*. 2011; 30(7):556–561. [PubMed: 21304419]
17. Brown ZA, Wald A, Morrow RA, Selke S, Zeh J, Corey L. Effect of serologic status and cesarean delivery on transmission rates of herpes simplex virus from mother to infant. *JAMA*. 2003; 289(2): 203–209. [PubMed: 12517231]
18. Arvin AM, Hensleigh PA, Prober CG, et al. Failure of Antepartum Maternal Cultures to Predict the Infant's Risk of Exposure to Herpes Simplex Virus at Delivery. *N Engl J Med*. 1986; 315(13):796–800. [PubMed: 3018565]
19. Hollier LM, Wendel GD. Third trimester antiviral prophylaxis for preventing maternal genital herpes simplex virus (HSV) recurrences and neonatal infection. *Cochrane Database Syst Rev*. 2008; CD004946(1)
20. Rouse DJ, Stringer JS. An appraisal of screening for maternal type-specific herpes simplex virus antibodies to prevent neonatal herpes. *AmJ Obstet Gynecol*. 2000; 183(2):400–406. [PubMed: 10942477]
21. Watts DH, Brown ZA, Money D, et al. A double-blind, randomized, placebo-controlled trial of acyclovir in late pregnancy for the reduction of herpes simplex virus shedding and cesarean delivery. *AmJ Obstet Gynecol*. 2003; 188(3):836–843. [PubMed: 12634667]
22. 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(1):73–83. [PubMed: 16327322]
23. Wald A, Link K. Risk of human immunodeficiency virus infection in herpes simplex virus type 2-seropositive persons: a meta-analysis. *J Infect Dis*. 2002; 185(1):45–52. [PubMed: 11756980]
24. Celum C, Wald A, Lingappa JR, et al. Acyclovir and transmission of HIV-1 from persons infected with HIV-1 and HSV-2. *N Engl J Med*. 2010; 362(5):427–439. [PubMed: 20089951]
25. Celum C, Wald A, Hughes J, et al. Effect of aciclovir on HIV-1 acquisition in herpes simplex virus 2 seropositive women and men who have sex with men: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2008; 371(9630):2109–2119. [PubMed: 18572080]
26. Watson-Jones D, Weiss HA, Rusizoka M, et al. Effect of herpes simplex suppression on incidence of HIV among women in Tanzania. *N Engl J Med*. 2008; 358(15):1560–1571. [PubMed: 18337596]
27. Zhu J, Hladik F, Woodward A, 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(8):886–892. [PubMed: 19648930]
28. Jing L, Schiffer JT, Chong TM, et al. CD4 T-cell memory responses to viral infections of humans show pronounced immunodominance independent of duration or viral persistence. *J Virol*. 2013; 87(5):2617–2627. [PubMed: 23255792]
29. Schiffer JT, Swan D, Al Sallaq R, et al. Rapid localized spread and immunologic containment define Herpes simplex virus-2 reactivation in the human genital tract. *Elife*. 2013; 2:e00288. [PubMed: 23606943]
30. Mertz GJ, Benedetti J, Ashley R, Selke SA, Corey L. Risk factors for the sexual transmission of genital herpes. *Ann Intern Med*. 1992; 116(3):197–202. [PubMed: 1309413]
31. Wald A, Zeh J, Selke S, et al. Reactivation of genital herpes simplex virus type 2 infection in asymptomatic seropositive persons. *N Engl J Med*. 2000; 342(12):844–850. [PubMed: 10727588]

32. Mark KE, Wald A, Magaret AS, et al. Rapidly cleared episodes of herpes simplex virus reactivation in immunocompetent adults. *J Infect Dis.* 2008; 198(8):1141–1149. [PubMed: 18783315]
33. Crespi CM, Cumberland WG, Wald A, Corey L, Blower S. Longitudinal study of herpes simplex virus type 2 infection using viral dynamic modelling. *Sex Transm Infect.* 2007; 83(5):359–364. [PubMed: 17475687]
34. Schiffer JT, Abu-Raddad L, Mark KE, et al. Frequent release of low amounts of herpes simplex virus from neurons: results of a mathematical model. *Sci Transl Med.* 2009; 1(7):7ra16.
- 35**. Corey L, Wald A. Maternal and neonatal herpes simplex virus infections. *N Engl J Med.* 2009; 361(14):1376–1385. Comprehensive review of the state of knowledge about maternal-infant transmission of HSV infection. [PubMed: 19797284]
36. Chentoufi AA, Kritzer E, Yu DM, Nesburn AB, Benmohamed L. Towards a rational design of an asymptomatic clinical herpes vaccine: the old, the new, and the unknown. *Clin Dev Immunol.* 2012; 2012:187585. [PubMed: 22548113]
37. Gantt S, Muller WJ. The immunologic basis for severe neonatal herpes disease and potential strategies for therapeutic intervention. *Clin Dev Immunol.* 2013; 2013:369172. [PubMed: 23606868]
- 38*. Muller WJ, Jones CA, Koelle DM. Immunobiology of herpes simplex virus and cytomegalovirus infections of the fetus and newborn. *Curr Immunol Rev.* 2010; 6(1):38–55. Excellent overview of the immunobiology of neonatal HSV infection and disease. [PubMed: 20467462]
39. Connolly SA, Jackson JO, Jardetzky TS, Longnecker R. Fusing structure and function: a structural view of the herpesvirus entry machinery. *Nat Rev Microbiol.* 2011; 9(5):369–381. [PubMed: 21478902]
40. Hummeler K, Tomassini N, Zajac B. Early events in herpes simplex virus infection: a radioautographic study. *J Virol.* 1969; 4(1):67–74. [PubMed: 4309102]
41. Milne RS, Nicola AV, Whitbeck JC, Eisenberg RJ, Cohen GH. Glycoprotein D receptor-dependent, low-pH-independent endocytic entry of herpes simplex virus type 1. *J Virol.* 2005; 79(11):6655–6663. [PubMed: 15890903]
42. Nicola AV, McEvoy AM, Straus SE. Roles for endocytosis and low pH in herpes simplex virus entry into HeLa and Chinese hamster ovary cells. *J Virol.* 2003; 77(9):5324–5332. [PubMed: 12692234]
43. Clement C, Tiwari V, Scanlan PM, Valyi-Nagy T, Yue BY, Shukla D. A novel role for phagocytosis-like uptake in herpes simplex virus entry. *J Cell Biol.* 2006; 174(7):1009–1021. [PubMed: 17000878]
44. Turner A, Bruun B, Minson T, Browne H. Glycoproteins gB, gD, and gHgL of herpes simplex virus type 1 are necessary and sufficient to mediate membrane fusion in a Cos cell transfection system. *J Virol.* 1998; 72(1):873–875. [PubMed: 9420303]
45. Nicola AV, Straus SE. Cellular and viral requirements for rapid endocytic entry of herpes simplex virus. *J Virol.* 2004; 78(14):7508–7517. [PubMed: 15220424]
46. Sodeik B, Ebersold MW, Helenius A. Microtubule-mediated transport of incoming herpes simplex virus 1 capsids to the nucleus. *J Cell Biol.* 1997; 136(5):1007–1021. [PubMed: 9060466]
47. Batterson W, Furlong D, Roizman B. Molecular genetics of herpes simplex virus. VIII. further characterization of a temperature-sensitive mutant defective in release of viral DNA and in other stages of the viral reproductive cycle. *J Virol.* 1983; 45(1):397–407. [PubMed: 6296445]
48. Steiner I, Kennedy PG. Herpes simplex virus latent infection in the nervous system. *J Neurovirol.* 1995; 1(1):19–29. [PubMed: 9222339]
49. Topp KS, Meade LB, LaVail JH. Microtubule polarity in the peripheral processes of trigeminal ganglion cells: relevance for the retrograde transport of herpes simplex virus. *J Neurosci.* 1994; 14(1):318–325. [PubMed: 8283239]
50. McNamee EE, Taylor TJ, Knipe DM. A dominant-negative herpesvirus protein inhibits intranuclear targeting of viral proteins: effects on DNA replication and late gene expression. *J Virol.* 2000; 74(21):10122–10131. [PubMed: 11024141]

51. Quinlan MP, Chen LB, Knipe DM. The intranuclear location of a herpes simplex virus DNA-binding protein is determined by the status of viral DNA replication. *Cell*. 1984; 36(4):857–868. [PubMed: 6323024]
52. Honess RW, Roizman B. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. *J Virol*. 1974; 14(1):8–19. [PubMed: 4365321]
53. Mettenleiter TC. Herpesvirus assembly and egress. *J Virol*. 2002; 76(4):1537–1547. [PubMed: 11799148]
54. Carneiro-Sampaio M, Coutinho A. Immunity to microbes: lessons from primary immunodeficiencies. *Infect Immun*. 2007; 75(4):1545–1555. [PubMed: 17283094]
55. Collins WJ, Johnson DC. Herpes simplex virus gE/gI expressed in epithelial cells interferes with cell-to-cell spread. *J Virol*. 2003; 77(4):2686–2695. [PubMed: 12552008]
56. Johnson DC, Webb M, Wisner TW, Brunetti C. Herpes simplex virus gE/gI sorts nascent virions to epithelial cell junctions, promoting virus spread. *J Virol*. 2001; 75(2):821–833. [PubMed: 11134295]
57. Posavad CM, Koelle DM, Shaughnessy MF, Corey L. Severe genital herpes infections in HIV-infected individuals with impaired herpes simplex virus-specific CD8+ cytotoxic T lymphocyte responses. *Proc Natl Acad Sci U S A*. 1997; 94(19):10289–10294. [PubMed: 9294203]
58. Zuckerman RA, Limaye AP. Varicella Zoster Virus (VZV) and Herpes Simplex Virus (HSV) in Solid Organ Transplant Patients. *Am J Transplant*. 2013; 13(Suppl 3):55–66. [PubMed: 23347214]
59. Koelle DM, Posavad CM, Barnum GR, Johnson ML, Frank JM, Corey L. Clearance of HSV-2 from recurrent genital lesions correlates with infiltration of HSV-specific cytotoxic T lymphocytes. *J Clin Invest*. 1998; 101(7):1500–1508. [PubMed: 9525993]
60. Zhu XP, Muhammad ZS, Wang JG, Lin W, Guo SK, Zhang W. HSV-2 vaccine: current status and insight into factors for developing an efficient vaccine. *Viruses*. 2014; 6(2):371–390. [PubMed: 24469503]
61. Halford WP. Antigenic breadth: a missing ingredient in HSV-2 subunit vaccines? *Expert Rev Vaccines*. 2014; 13(6):691–710. [PubMed: 24837838]
62. Dervillez X, Qureshi H, Chentoufi AA, et al. Asymptomatic HLA-A*02:01-restricted epitopes from herpes simplex virus glycoprotein B preferentially recall polyfunctional CD8+ T cells from seropositive asymptomatic individuals and protect HLA transgenic mice against ocular herpes. *J Immunol*. 2013; 191(10):5124–5138. [PubMed: 24101547]
- 63**. Zhu J, Peng T, Johnston C, et al. Immune surveillance by CD8alphaalpha+ skin-resident T cells in human herpes virus infection. *Nature*. 2013; 497(7450):494–497. Novel insights into immune effectors responsible for control of HSV reactivation, shedding and disease that may lead to new and more effective vaccine strategies in the future. [PubMed: 23657257]
64. Khodai T, Chappell D, Christy C, et al. Single and combination herpes simplex virus type 2 glycoprotein vaccines adjuvanted with CpG oligodeoxynucleotides or monophosphoryl lipid A exhibit differential immunity that is not correlated to protection in animal models. *Clin Vaccine Immunol*. 2011; 18(10):1702–1709. [PubMed: 21852545]
65. de Veer M, Meeusen E. New developments in vaccine research--unveiling the secret of vaccine adjuvants. *Discov Med*. 2011; 12(64):195–204. [PubMed: 21955847]
66. Gebril A, Alsaadi M, Acevedo R, Mullen AB, Ferro VA. Optimizing efficacy of mucosal vaccines. *Expert Rev Vaccines*. 2012; 11(9):1139–1155. [PubMed: 23151169]
- 67*. Dasgupta G, BenMohamed L. Of mice and not humans: how reliable are animal models for evaluation of herpes CD8(+)-T cell-epitopes-based immunotherapeutic vaccine candidates? *Vaccine*. 2011; 29(35):5824–5836. Valuable overview of the pros and cons of animal models in predicting responses to human trials of HSV vaccines. [PubMed: 21718746]
- 68*. Valencia F, Veselenak RL, Bourne N. In vivo evaluation of antiviral efficacy against genital herpes using mouse and Guinea pig models. *Methods Mol Biol*. 2013; 1030:315–326. Excellent overview of the guinea pig HSV model, the only small animal model for recurrent genital HSV infection. [PubMed: 23821278]
69. Thornton B, Griffiths JB, Walkland A. Herpes simplex vaccine using cell membrane associated antigen in an animal model. *Dev Biol Stand*. 1981; 50:201–206. [PubMed: 6281105]

70. Hsiung GD, Mayo DR, Lucia HL, Landry ML. Genital herpes: pathogenesis and chemotherapy in the guinea pig model. *Rev Infect Dis.* 1984; 6(1):33–50. [PubMed: 6324317]
71. Stanberry LR. Evaluation of herpes simplex virus vaccines in animals: the guinea pig vaginal model. *Rev Infect Dis.* 1991; 13(Suppl 11):S920–923. [PubMed: 1664129]
72. Zaia JA, Palmer EL, Feorino PM. Humoral and cellular immune responses to an envelope-associated antigen of herpes simplex virus. *J Infect Dis.* 1975; 132(6):660–666. [PubMed: 172562]
73. Koszinowski U, Bandlow G. Induction and in Vitro demonstration of cellular immunity to DNA and RNA viruses in guinea-pigs. *Clin Exp Immunol.* 1975; 20(1):143–154. [PubMed: 173485]
74. Benda R, Dbaly V. Immunogenic properties of formalin herpes antigen prepared from cell cultures. *J Hyg Epidemiol Microbiol Immunol.* 1973; 17(2):237–249.
75. Kavaklova L, Dundarov S, Andonov P, Bakalov B, Dundarova D, Brodvarova I. Preparation and efficacy of antiherpes type 1 and 2 subunit vaccines. *Acta Virol.* 1986; 30(5):402–410. [PubMed: 2878588]
76. Kutinova L, Slichtova V, Vonka V. Immunogenicity of subviral herpes simplex virus preparations. I. Formation of neutralizing antibodies in different animal species after administration of herpes simplex virus solubilized antigens. *Arch Virol.* 1979; 61(1-2):141–147. [PubMed: 229789]
77. Berman PW, Gregory T, Crase D, Lasky LA. Protection from genital herpes simplex virus type 2 infection by vaccination with cloned type 1 glycoprotein D. *Science.* 1985; 227(4693):1490–1492. [PubMed: 2983428]
78. Berman PW, Vogt PE, Gregory T, Lasky LA, Kern ER. Efficacy of recombinant glycoprotein D subunit vaccines on the development of primary, recurrent, and latent genital infections with herpes simplex virus type 2 in guinea pigs. *J Infect Dis.* 1988; 157(5):897–902. [PubMed: 2834467]
79. Stanberry LR, Bernstein DI, Burke RL, Pacht C, Myers MG. Vaccination with recombinant herpes simplex virus glycoproteins: protection against initial and recurrent genital herpes. *J Infect Dis.* 1987; 155(5):914–920. [PubMed: 3031173]
80. Byars NE, Fraser-Smith EB, Pecyk RA, et al. Vaccinating guinea pigs with recombinant glycoprotein D of herpes simplex virus in an efficacious adjuvant formulation elicits protection against vaginal infection. *Vaccine.* 1994; 12(3):200–209. [PubMed: 8165851]
81. Quenelle DC, Collins DJ, Rice TL, Prichard MN, Marciani DJ, Kern ER. Effect of an immune enhancer, GPI-0100, on vaccination with live attenuated herpes simplex virus (HSV) type 2 or glycoprotein D on genital HSV-2 infections of guinea pigs. *Antiviral Res.* 2008; 80(2):223–224. [PubMed: 18573279]
82. Bourne N, Milligan GN, Stanberry LR, Stegall R, Pyles RB. Impact of immunization with glycoprotein D2/AS04 on herpes simplex virus type 2 shedding into the genital tract in guinea pigs that become infected. *J Infect Dis.* 2005; 192(12):2117–2123. [PubMed: 16288376]
83. Burke RL, Goldbeck C, Ng P, Stanberry L, Ott G, Van Nest G. The influence of adjuvant on the therapeutic efficacy of a recombinant genital herpes vaccine. *J Infect Dis.* 1994; 170(5):1110–1119. [PubMed: 7963702]
84. Sanchez-Pescador L, Burke RL, Ott G, Van Nest G. The effect of adjuvants on the efficacy of a recombinant herpes simplex virus glycoprotein vaccine. *J Immunol.* 1988; 141(5):1720–1727. [PubMed: 2842401]
85. Bernstein DI, Earwood JD, Bravo FJ, et al. Effects of herpes simplex virus type 2 glycoprotein vaccines and CLDC adjuvant on genital herpes infection in the guinea pig. *Vaccine.* 2011; 29(11):2071–2078. [PubMed: 21238569]
86. Dropulic LK, Cohen JI. The challenge of developing a herpes simplex virus 2 vaccine. *Expert Rev Vaccines.* 2012; 11(12):1429–1440. [PubMed: 23252387]
87. O'Hagan D, Goldbeck C, Ugozzoli M, Ott G, Burke RL. Intranasal immunization with recombinant gD2 reduces disease severity and mortality following genital challenge with herpes simplex virus type 2 in guinea pigs. *Vaccine.* 1999; 17(18):2229–2236. [PubMed: 10403590]
88. Gallichan WS, Woolstencroft RN, Guarasci T, McCluskie MJ, Davis HL, Rosenthal KL. Intranasal immunization with CpG oligodeoxynucleotides as an adjuvant dramatically increases IgA and protection against herpes simplex virus-2 in the genital tract. *J Immunol.* 2001; 166(5):3451–3457. [PubMed: 11207303]

89. Skoberne M, Cardin R, Lee A, 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(7): 3930–3942. [PubMed: 23365421]
90. Awasthi S, Lubinski JM, Shaw CE, et al. Immunization with a vaccine combining herpes simplex virus 2 (HSV-2) glycoprotein C (gC) and gD subunits improves the protection of dorsal root ganglia in mice and reduces the frequency of recurrent vaginal shedding of HSV-2 DNA in guinea pigs compared to immunization with gD alone. *J Virol.* 2011; 85(20):10472–10486. [PubMed: 21813597]
91. Wizel B, Persson J, Thorn K, Nagy E, Harandi AM. Nasal and skin delivery of IC31((R))-adjuvanted recombinant HSV-2 gD protein confers protection against genital herpes. *Vaccine.* 2012; 30(29):4361–4368. [PubMed: 22682292]
92. Manservigi R, Boero A, Argnani R, et al. Immunotherapeutic activity of a recombinant combined gB-gD-gE vaccine against recurrent HSV-2 infections in a guinea pig model. *Vaccine.* 2005; 23(7):865–872. [PubMed: 15603886]
93. McClements WL, Armstrong ME, Keys RD, Liu MA. The prophylactic effect of immunization with DNA encoding herpes simplex virus glycoproteins on HSV-induced disease in guinea pigs. *Vaccine.* 1997; 15(8):857–860. [PubMed: 9234533]
94. Bourne N, Stanberry LR, Bernstein DI, Lew D. DNA immunization against experimental genital herpes simplex virus infection. *J Infect Dis.* 1996; 173(4):800–807. [PubMed: 8603957]
95. McClements WL, Armstrong ME, Keys RD, Liu MA. Immunization with DNA vaccines encoding glycoprotein D or glycoprotein B, alone or in combination, induces protective immunity in animal models of herpes simplex virus-2 disease. *Proc Natl Acad Sci U S A.* 1996; 93(21):11414–11420. [PubMed: 8876149]
96. Bourne N, Milligan GN, Schleiss MR, Bernstein DI, Stanberry LR. DNA immunization confers protective immunity on mice challenged intravaginally with herpes simplex virus type 2. *Vaccine.* 1996; 14(13):1230–1234. [PubMed: 8961510]
97. Fotouhi F, Soleimanjahi H, Roostae MH, Behzadian F. Enhancement of protective humoral immune responses against Herpes simplex virus-2 in DNA-immunized guinea-pigs using protein boosting. *FEMS Immunol Med Microbiol.* 2008; 54(1):18–26. [PubMed: 18647350]
98. Bernstein DI, Tepe ER, Mester JC, Arnold RL, Stanberry LR, Higgins T. Effects of DNA immunization formulated with bupivacaine in murine and guinea pig models of genital herpes simplex virus infection. *Vaccine.* 1999; 17(15-16):1964–1969. [PubMed: 10217595]
99. Shlapobersky M, Marshak JO, Dong L, et al. Vaxfectin-adjuvanted plasmid DNA vaccine improves protection and immunogenicity in a murine model of genital herpes infection. *J Gen Virol.* 2012; 93(Pt 6):1305–1315. [PubMed: 22398318]
100. Veselenak RL, Shlapobersky M, Pyles RB, Wei Q, Sullivan SM, Bourne N. A Vaxfectin((R))-adjuvanted HSV-2 plasmid DNA vaccine is effective for prophylactic and therapeutic use in the guinea pig model of genital herpes. *Vaccine.* 2012; 30(49):7046–7051. [PubMed: 23041125]
101. Bright H, Perez DL, Christy C, et al. The efficacy of HSV-2 vaccines based on gD and gB is enhanced by the addition of ICP27. *Vaccine.* 2012; 30(52):7529–7535. [PubMed: 23103198]
102. Morello CS, Kraynyak KA, Levinson MS, Chen Z, Lee KF, Spector DH. Inactivated HSV-2 in MPL/alum adjuvant provides nearly complete protection against genital infection and shedding following long term challenge and rechallenge. *Vaccine.* 2012; 30(46):6541–6550. [PubMed: 22947141]
103. Nelson J, Rodriguez S, Finlayson N, Williams J, Carnes A. Antibiotic-free production of a herpes simplex virus 2 DNA vaccine in a high yield cGMP process. *Hum Vaccin Immunother.* 2013; 9(10)
- 104*. Halford WP, Puschel R, Rakowski B. Herpes simplex virus 2 ICP0 mutant viruses are avirulent and immunogenic: implications for a genital herpes vaccine. *PLoS One.* 2010; 5(8):e12251. Dramatic improvement in protection conferred by attenuated ICP0 mutant virus vaccines compared to adjuvanted glycoproteins provide support for this approach in the clinic, regulatory hurdles notwithstanding. [PubMed: 20808928]

105. Halford WP, Geltz J, Gershburg E. Pan-HSV-2 IgG antibody in vaccinated mice and guinea pigs correlates with protection against herpes simplex virus 2. *PLoS One*. 2013; 8(6):e65523. [PubMed: 23755244]
106. Shin H, Iwasaki A. A vaccine strategy that protects against genital herpes by establishing local memory T cells. *Nature*. 2012; 491(7424):463–467. [PubMed: 23075848]
107. Belshe RB, Leone PA, Bernstein DI, et al. Efficacy results of a trial of a herpes simplex vaccine. *N Engl J Med*. 2012; 366(1):34–43. [PubMed: 22216840]
108. Leroux-Roels G, Clement F, Vandepapeliere P, Fourneau M, Heineman TC, Dubin G. Immunogenicity and safety of different formulations of an adjuvanted glycoprotein D genital herpes vaccine in healthy adults: A double-blind randomized trial. *Hum Vaccin Immunother*. 2013; 9(6)
- 109*. Stanberry LR, Spruance SL, Cunningham AL, et al. Glycoprotein-D-adjuvant vaccine to prevent genital herpes. *N Engl J Med*. 2002; 347(21):1652–1661. The only clinical trial demonstrating an impact of a HSV vaccine on acquisition of primary infection also confirmed a protective role for pre-existing HSV-1 immunity against acquisition of HSV-2 infection. [PubMed: 12444179]
110. Wald A, Koelle DM, Fife K, et al. Safety and immunogenicity of long HSV-2 peptides complexed with rhHsc70 in HSV-2 seropositive persons. *Vaccine*. 2011; 29(47):8520–8529. [PubMed: 21945262]
111. Da Costa X, Kramer MF, Zhu J, Brockman MA, Knipe DM. Construction, phenotypic analysis, and immunogenicity of a UL5/UL29 double deletion mutant of herpes simplex virus 2. *J Virol*. 2000; 74(17):7963–7971. [PubMed: 10933704]
112. Dudek T, Mathews LC, Knipe DM. Disruption of the U(L)41 gene in the herpes simplex virus 2 dl5-29 mutant increases its immunogenicity and protective capacity in a murine model of genital herpes. *Virology*. 2008; 372(1):165–175. [PubMed: 18006033]
113. Reszka NJ, Dudek T, Knipe DM. Construction and properties of a herpes simplex virus 2 dl5-29 vaccine candidate strain encoding an HSV-1 virion host shutoff protein. *Vaccine*. 2010; 28(15):2754–2762. [PubMed: 20117270]
114. Hoshino Y, Pesnicak L, Dowdell KC, et al. Comparison of immunogenicity and protective efficacy of genital herpes vaccine candidates herpes simplex virus 2 dl5-29 and dl5-29-41L in mice and guinea pigs. *Vaccine*. 2008; 26(32):4034–4040. [PubMed: 18565628]
115. Hoshino Y, Pesnicak L, Dowdell KC, et al. Protection from herpes simplex virus (HSV)-2 infection with replication-defective HSV-2 or glycoprotein D2 vaccines in HSV-1-seropositive and HSV-1-seronegative guinea pigs. *J Infect Dis*. 2009; 200(7):1088–1095. [PubMed: 19702506]
116. McLean CS, Ni Challanain D, Duncan I, Boursnell ME, Jennings R, Inglis SC. Induction of a protective immune response by mucosal vaccination with a DISC HSV-1 vaccine. *Vaccine*. 1996; 14(10):987–992. [PubMed: 8873393]
117. McLean CS, Erturk M, Jennings R, et al. Protective vaccination against primary and recurrent disease caused by herpes simplex virus (HSV) type 2 using a genetically disabled HSV-1. *J Infect Dis*. 1994; 170(5):1100–1109. [PubMed: 7963701]
118. Boursnell ME, Entwisle C, Blakeley D, et al. A genetically inactivated herpes simplex virus type 2 (HSV-2) vaccine provides effective protection against primary and recurrent HSV-2 disease. *J Infect Dis*. 1997; 175(1):16–25. [PubMed: 8985191]
119. de Bruyn G, Vargas-Cortez M, Warren T, et al. A randomized controlled trial of a replication defective (gH deletion) herpes simplex virus vaccine for the treatment of recurrent genital herpes among immunocompetent subjects. *Vaccine*. 2006; 24(7):914–920. [PubMed: 16213066]
120. Posavad CM, Remington M, Mueller DE, et al. Detailed characterization of T cell responses to herpes simplex virus-2 in immune seronegative persons. *J Immunol*. 2010; 184(6):3250–3259. [PubMed: 20164419]
121. Dollery SJ, Delboy MG, Nicola AV. Low pH-induced conformational change in herpes simplex virus glycoprotein B. *J Virol*. 2010; 84(8):3759–3766. [PubMed: 20147407]
122. Stampfer SD, Lou H, Cohen GH, Eisenberg RJ, Heldwein EE. Structural basis of local, pH-dependent conformational changes in glycoprotein B from herpes simplex virus type 1. *J Virol*. 2010; 84(24):12924–12933. [PubMed: 20943984]

123. Dasgupta G, Chentoufi AA, Kalantari M, et al. Immunodominant “asymptomatic” herpes simplex virus 1 and 2 protein antigens identified by probing whole-ORFome microarrays with serum antibodies from seropositive asymptomatic versus symptomatic individuals. *J Virol.* 2012; 86(8): 4358–4369. [PubMed: 22318137]
124. Dervillez X, Gottimukkala C, Kabbara KW, et al. Future of an “Asymptomatic” T-cell Epitope-Based Therapeutic Herpes Simplex Vaccine. *Future Virol.* 2012; 7(4):371–378. [PubMed: 22701511]
125. St Leger AJ, Jeon S, Hendricks RL. Broadening the repertoire of functional herpes simplex virus type 1-specific CD8+ T cells reduces viral reactivation from latency in sensory ganglia. *J Immunol.* 2013
- 126*. Jing L, Haas J, Chong TM, 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(2): 654–673. Elegant approach to identification of candidate vaccine T-cell antigens for a HSV-1 vaccine. [PubMed: 22214845]
127. Takahashi M, Otsuka T, Okuno Y, Asano Y, Yazaki T. Live vaccine used to prevent the spread of varicella in children in hospital. *Lancet.* 1974; 2(7892):1288–1290. [PubMed: 4139526]
128. Gershon AA, Chen J, Davis L, et al. Latency of varicella zoster virus in dorsal root, cranial, and enteric Ganglia in vaccinated children. *Trans Am Clin Climatol Assoc.* 2012; 123:17–35. [PubMed: 23303966]
129. Abendroth A, Kinchington PR, Slobedman B. Varicella zoster virus immune evasion strategies. *Curr Top Microbiol Immunol.* 2010; 342:155–171. [PubMed: 20563710]
130. Johnston C, Koelle DM, Wald A. HSV-2: in pursuit of a vaccine. *J Clin Invest.* 2011; 121(12): 4600–4609. [PubMed: 22133885]

Key Issues

- Antivirals for HSV infection have not significantly altered the epidemiology of HSV infections; therefore, vaccination is required to reduce morbidity and mortality.
- Significant differences in the natural history of Varicella-zoster virus infection compared with HSV, particularly the lack of viral protein expression during HSV latency, may inform the development of future candidate HSV vaccines. The short incubation period for HSV as well as the limited viremic stage of primary infection further complicate vaccine development; mucosal immunity may be more important than systemic IgG response.
- Protective adaptive responses in immune-seronegative and asymptomatic subjects seem to be skewed towards early HSV proteins. Future candidate vaccines should include some of these “protective” and “asymptomatic” antigens.
- Antibodies induced by experimental vaccines that are found to have neutralizing activity *in vitro* may have little or no effect on viral neutralization *in vivo*, possibly because of inadequate tissue-specific concentrations, pH-dependency of certain antigens, and the inherent inability of antibody to prevent cell-to-cell spread of progeny virus during lytic replication.
- Cell mediated immunity is essential for control of HSV infection and may in some instances develop in the absence of a humoral response. However, tools for assessing the effectiveness of CMI responses are infrequently employed in vaccine research and little standardization and consensus opinion on correlates of infection has been established.
- Further development in adjuvant technology may increase our ability to induce innate immune mechanisms, CMI, and responses targeted to key mucosal sites.
- Innovative work dissecting and characterizing local resident T cell populations in individuals with excellent disease control may lead to novel future vaccine approaches.

Table 1

Recent and Ongoing Clinical Trials

Vaccine	Population	Adjuvant	Vaccination Schedule	Outcomes	Results
Recombinant subunit					
HERPEVAC (GSK) truncated HSV-2 gD	Sero-discordant monogamous heterosexual couples HSV-1 and -2 negative women	Alum and MPL	0, 1, 6 months	Prevention of primary infection	<ul style="list-style-type: none"> Moderate efficacy against genital herpes in women (seronegative for HSV-1 and HSV-2) but not men [109] Slight protection from HSV-1 infection and disease; no protection from HSV-2 nor reduction in viral shedding [107]
GEN-003 (Genocoe) truncated HSV-2 gD and ICP4	Subjects with moderate to severe HSV-2 infection	Matrix M™	0, 3, 6 weeks	Safety, tolerability, reduced viral shedding	<ul style="list-style-type: none"> Ongoing [ClinicalTrials.gov identifier NCT01667341]
HerpV (Agenus) peptides from 22 HSV-2 proteins non-covalently linked to HSC70	HSV-2 seropositives	Stimulon®	1 to 3 doses	Safety, tolerability, immunogenicity	<ul style="list-style-type: none"> Broad induction of humoral and cell mediated immunity Transient humoral responses to HSC70 Ongoing [ClinicalTrials.gov identifier NCT01687595]
VCL-HM01 (Vical; Phase 2; monovalent) and HB01 (Phase 1; bivalent) DNA vaccines	HSV-2 seropositives 2-9 recurrences/year 18-50 years of age	Vaxfectin®	3 doses Dose escalation	Safety, tolerability, viral shedding, recurrence rate, T-cell response	<ul style="list-style-type: none"> Ongoing [ClinicalTrials.gov identifier NCT02030301]
Live, replication deficient					
HSV529 (Sanofi Pasteur)	HSV-1 and HSV-2 infected and uninfected subjects		0, 1, 6 months	Safety, tolerability, immunogenicity	<ul style="list-style-type: none"> Ongoing [ClinicalTrials.gov identifier NCT01915212]

GSK, GlaxoSmithKline; MPL, 3-O-deacylated-monophosphoryl lipid A