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The challenge of developing a herpes simplex virus 2 vaccine

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Abstract

HSV infections are prevalent worldwide. A vaccine to prevent genital herpes would have a significant impact on this disease. Several vaccines have shown promise in animal models; however, so far these have not been successful in human clinical studies. Prophylactic HSV vaccines to prevent HSV infection or disease have focused primarily on eliciting antibody responses. Potent antibody responses are needed to result in sufficiently high levels of virus-specific antibody in the genital tract. Therapeutic vaccines that reduce recurrences need to induce potent T-cell responses at the site of infection. With the increasing incidence of HSV-1 genital herpes, an effective herpes vaccine should protect against both HSV-1 and HSV-2. Novel HSV vaccines, such as replication-defective or attenuated viruses, have elicited humoral and cellular immune responses in preclinical studies. These vaccines and others hold promise in future clinical studies.

Keywords

animal models; genital herpes; herpes simplex virus; immune response; prophylactic vaccine; therapeutic vaccine

Pathogenesis

HSV-2 contains a double-stranded DNA genome of about 152 kb pairs that encodes at least 84 proteins. The viral genome is enclosed in an icosahedral capsid surrounded by an envelope composed of a lipid bilayer and at least 13 viral glycoproteins. Transmission of the virus requires intimate contact between a person excreting HSV and a susceptible person. The portal of entry for HSV-2 infection is the genital or, less frequently, oral mucosa or areas of abraded skin. The virus replicates in epithelial and mucosal cells with subsequent infection of sensory nerve endings. The virus is then transported to dorsal root or cranial nerve ganglia where latency is established. Primary genital infection results in painful vesicles and ulcers, and can be complicated by fever, local lymphadenopathy, dysuria, paresthesias and aseptic meningitis. HSV-2 can be spread to the neonate during delivery,

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resulting in disseminated infection in the newborn. The virus can reactivate from latency to cause recurrent disease at mucosal and epithelial surfaces, resulting in genital herpes or herpes labialis; shedding from these sites can spread virus to susceptible contacts. The observation that HSV-2 frequently reactivates in the presence of virus-specific neutralizing antibody and T-cell responses implies that a therapeutic vaccine based on the development of the level of immune responses observed during natural infection might be insufficient to prevent disease associated with reactivation.

Rationale for the development of an HSV-2 vaccine

Worldwide, more than 500 million people are estimated to be infected with HSV-2 [1]. While genital herpes can cause substantial morbidity including psychological distress, the majority of persons infected with HSV-2 are asymptomatic and unaware of their recurrences [2]. Asymptomatic shedding facilitates the spread of HSV-2 throughout the population. Disseminated HSV-2 infection of neonates, which can result from exposure to HSV during delivery, has a mortality rate up to 85% if untreated [3]. Seventy five percent of these babies develop encephalitis and nearly all of them have significant neurological sequelae [4]. HSV-2 causes severe, sometimes refractory disease in patients with HIV/AIDS and other immunocompromising conditions [5,6]. In addition to severe genital and anal disease, HSV-2 can cause visceral disease and infect the spinal cord in patients with HIV/AIDS [7]. Epidemiologic studies have demonstrated that HSV-2 seropositive persons have a two- to fourfold increased risk of acquiring HIV-1 infection [8]. In addition, HSV increases the risk of HIV transmission if the source partner is infected with HSV-2 [8,9]. While antiviral drugs suppress recurrences of genital HSV disease and decrease shedding, they reduce HSV transmission rates to susceptible partners by only about 50% [10].

Animal models used for HSV-2 vaccine development

Animal models are critical for development of HSV-2 vaccines. The principal animals used are mice and guinea pigs, although cotton rats and owl monkeys have been used to a limited extent (Table 1). Most candidate HSV-2 vaccine studies are initially tested in mice. Mice infected vaginally with HSV-2, after treatment with medroxyprogesterone, develop acute disease, shed virus during acute infection, and establish latent infection in the associated dorsal root ganglia. Humoral and cellular immune responses to the vaccine can be measured easily. Spontaneous reactivation does not occur, but can be induced with UV irradiation or hyperthermia [11,12]. Therefore, in mice, it is not possible to evaluate the impact of vaccination on recurrent disease. In addition, intravaginal inoculation of virus often results in spread of the virus to the sacral ganglia resulting in urinary retention, hind limb paralysis, and death of the mouse. The use of inbred strains or HLA transgenic mice, allows one to determine the contribution of individual components of the immune system to control infection or to determine human T-cell epitopes, respectively.

Since guinea pigs experience spontaneous recurrences of genital herpes, they serve as a useful model for testing whether prophylactic vaccines prevent acute disease and recurrences, as well as whether therapeutic vaccines reduce recurrences in animals that were previously infected [13,14]. Guinea pigs shed virus during acute infection, have recurrences that are HSV-2 DNA PCR positive (less frequently HSV-2 culture positive) and develop latent infection of sacral ganglia after intravaginal infection. Antibody responses to the virus can be measured but cellular immunity has not been well characterized, owing to lack of immune reagents.

Intravaginal inoculation of cotton rats with HSV-2 results in acute infections that do not require prior treatment with medroxy-progesterone [15]. Spontaneous recurrences occur.

Unfortunately, these animals are difficult to work with and, as with guinea pigs, reagents for measuring immunity are limited.

HSV-2 infection of rhesus macaques treated with medroxy-progesterone results in genital lesions in up to 10% of animals; thus, the rhesus model is not practical for vaccine studies [16]. By contrast, owl monkeys (*Aotus trivirgatus*) develop fatal infections after inoculation with HSV-2 [17]. These animals have been used to demonstrate the safety of live attenuated vaccines [18].

While candidate HSV-2 glycoprotein vaccines have been protective in mice and guinea pigs, these vaccines have all failed in human clinical trials. A number of reasons may explain these findings. First, HSV has coevolved with humans over millions of years, and major histocompatibility complex alleles are different in humans than in other animals. Viral proteins are likely to have changed their sequences to limit their recognition by the human immune system, which is different from the murine immune system, especially innate and mucosal immunity. In addition, the distribution of types of immune cells in the genital mucosa of inbred mice differs from that of outbred human populations. Second, the virus encodes a number of immune evasion genes. Many of these have species-specific effects. For example, HSV ICP47, which downregulates MHC class I by inhibiting TAP, has significantly less activity in mouse than in human cells [19]. Glycoprotein E binds the Fc domain of human IgG and blocks its activity, but does not bind the Fc domain of mouse or guinea pig IgG [20]. Third, animals are usually given a single challenge dose, while humans are challenged with virus during multiple exposures. While this reduces the required number of animals to show effectiveness of vaccines and improves statistical power, it differs markedly from human infection, in which most exposures to HSV-2 do not result in clinical disease. As such, vaccines that are ineffective in animals owing to an overwhelming titer of challenge virus might be effective in humans. Fourth, animals are usually challenged at the peak of their immune response to a vaccine, while humans would become infected months to years after vaccination. Fifth, viruses used to challenge animals are usually amplified in cell culture where they may undergo attenuating mutations [21]. Humans are infected with viruses that have not been passaged in culture.

Viral proteins as immunogens

Neutralizing antibodies

HSV contains 13 glycoproteins in its envelope that function in virus attachment, entry and fusion with cells. The most abundantly expressed glycoprotein on the virion and on the surface of virus-infected cells is glycoprotein D (gD). gD is a major target of neutralizing antibodies [22]. HSV-2 gD shares 98–99% amino acid identity among different HSV-2 strains and 82–88% amino acid identity with gD of different HSV-1 strains. gD is also a target of antibody-dependent cellular cytotoxicity, as well as of CD4⁺ and CD8⁺ T-cell responses [23,24]. Glycoproteins gB, gC and gE also trigger humoral and cellular immune responses. HSV-specific antibody is important for preventing neonatal HSV-2 disease [25]. Women who were previously infected before childbirth transmit HSV antibody across the placenta and are less likely to have infants with neonatal HSV-2 disease than women with acute HSV-2 infection at the time of childbirth, before antibody can be transmitted to the neonate [26]. A gD subunit vaccine that induced antibody to glycoprotein D at titers greater than those achieved with natural infection did not protect humans from HSV-2 genital disease [27]. This glycoprotein subunit vaccine may have been inadequate because the elicited immune responses were limited to a few epitopes, the duration of the neutralizing antibody response was very transient and the vaccine did not induce virus-specific CD8⁺ T cells [28].

T-cell responses

Activated CD4⁺ T cells are present early in HSV lesions in humans and later CD8⁺ T cells traffic to lesions [29,30]. CD8⁺ T cells persist in lesions more than 2 months after lesions heal, and are associated with clearance of virus [30,31]. In mice, the rate of reactivation is dependent upon the number of infiltrating CD8⁺ T cells and the number of viral genomes in latently infected ganglia [31,32]. CD8⁺ T cells are associated with clearance of virus and control reactivation in ganglia of mice [32,33].

HSV-2-specific CD8⁺ T cells recognize a large array of HSV proteins. In a study of 48 viral proteins in 21 HSV-2 seropositive subjects, the most frequent proteins recognized by CD8⁺ T cells (in order from highest to lowest) were UL39 (the viral large subunit of ribonucleotide reductase), UL25 capsid protein, UL27 (gB), ICP0 (an immediate-early protein), UL46 tegument protein, UL47 tegument protein, UL19 capsid protein, UL36 tegument protein, UL49 tegument protein and UL26 tegument protein [34]. HSV-2 infected persons recognized between three and 46 of 48 viral proteins, with a median of 11 proteins per person. A second study evaluated 14 viral proteins in 55 HSV-2 seropositive persons and found that the most frequently recognized HSV-2 proteins were UL39, ICP0, UL49, UL19, UL25, UL46, UL27, UL47, UL29 (single-stranded DNA binding protein), ICP27 (an immediate-early protein), UL11 tegument protein, UL35 capsid protein and US6 (gD) [35]. The HSV-2 specific CD8⁺ T cells more often expressed IFN- γ than IL-2 or TNF- α , and more often expressed granzyme B than perforin. When CD8⁺ T cells were sorted for cells expressing the cutaneous lymphocyte-associated antigen (a skin homing associated receptor), a more limited number of viral proteins were recognized [36]. Analysis of HSV-2-specific CD8⁺ T cells from genital lesions showed that UL47, UL49 and ICP0 were recognized by the cells [37], while virus-specific CD4⁺ T cells from genital lesions recognized UL49, UL50 (the viral dUTPase) and the UL21 tegument protein [38].

A study comparing T-cell responses present in the blood of HSV-2 immune but seronegative persons with HSV-2 seropositive persons showed that the former had T cells predominantly directed against UL39 (55% of persons responding), and the immediate-early proteins ICP4 (35%) and ICP0 (25%), followed by UL19 (15%) and UL29 (15%). By contrast, HSV-2 seropositive persons had T-cell responses to UL39 (50%), gD (43%), ICP0 (40%), ICP4 (38%), UL46 (25%), UL49 (28%) and gB (20%) [39]. This suggests that T-cell responses to UL39 and immediate-early proteins (rather than glycoprotein or tegument proteins) might be more important to protect against HSV-2 infection.

Innate immune responses are also important for control of HSV-2. IFN- α and IFN- β inhibit HSV replication [40,41]. Mutations in UNC-93B (UNC-93 homolog B1), TLR3, TRAF3 and TRIF are associated with childhood herpes simplex encephalitis [42]. Patients with mutations in STAT1, Tyk2, TRAF3, NEMO, as well as those with NK cell deficiency, have been reported with severe HSV infections [43].

At present, it is not known which immune responses are required to protect against HSV-2 disease. The observation that persons can be infected with more than one strain of HSV-2 suggests that vaccines may not completely protect against infection [44,45]. However, it is possible that these persons were initially infected with multiple strains rather than sequentially infected with different strains. Vaccines may protect against disease, reduce virus shedding or reduce the level of infection such that rates of transmission, neonatal disease and other symptomatic diseases are reduced.

History of HSV-2 vaccines tested in clinical studies

Early studies with inactivated virus & virus components from inactivated virus-infected cells

Attempts at developing an HSV vaccine date back to the 1930s. The vaccines were evaluated either as prophylactic or therapeutic vaccines. Prophylactic vaccines prevent acquisition of HSV-2 infection and/or prevent clinical disease, whereas therapeutic vaccines minimize disease severity and/or prevent HSV recurrences in persons who are already infected. Early research was hampered by crude vaccine preparations and poorly designed clinical studies. From the 1940s to the 1960s, vaccine virus was grown in embryonated eggs and later in cell cultures, and inactivated by UV irradiation, heat or chemicals. The first randomized, double-blind, placebo-controlled clinical trial of an HSV vaccine was conducted by Kern and Schiff in 1964 [46]. Formaldehyde-inactivated whole virus vaccine was administered to patients with recurrent HSV. The decrease in the number of recurrences was similar between the vaccine and placebo recipients. Other inactivated HSV vaccines have been tested in humans; however, they were not tested in double-blind, placebo-controlled trials and, therefore, the efficacy of the vaccines cannot be determined.

Owing to difficulties in ensuring that the entire virus is inactivated, vaccine developers isolated HSV components from detergent extracts of inactivated virus-infected cells. One such vaccine composed of DNA-free viral antigens extracted from HSV-infected human cells was tested in a double-blind study of 42 subjects experiencing frequent recurrences of HSV-1 or HSV-2 disease [47]. This vaccine was highly immunogenic in laboratory animals; however, it was poorly immunogenic in vaccinated subjects and there was no significant difference in reduction of clinical disease between the control and vaccine recipients.

In 1990, Mertz *et al.* reported the results of a double-blind, placebo-controlled trial of a vaccine containing HSV-2 gB, gC, gD, gE and gG derived from virus-infected chick embryo fibroblasts [48]. The vaccine failed to protect HSV-2 seronegative recipients, whose partners had documented recurrent genital herpes, from developing HSV-2 genital disease. Antibody titers to HSV-2 gD and gB were very low compared with the partners with recurrent genital herpes.

Skinner *et al.* developed a cell culture-derived vaccine composed of a mixture of HSV-1 glycoproteins inactivated with formalin and extracted with detergents [49]. A multicenter, placebo-controlled trial of this vaccine in patients with frequently recurring genital herpes revealed that the vaccine did not significantly decrease the frequency of genital herpes recurrences in women at 3 and 6 months after vaccination [49]. However, the severity of recurrences was significantly decreased as defined by a reduced number of lesions and reduced symptoms per recurrence. The vaccine induced both neutralizing antibody and cellular immunity to HSV-1.

Prophylactic vaccines

Recombinant glycoprotein subunit vaccines

Glycoprotein vaccines consist of one or more glycoproteins combined with adjuvants that boost their immunity. gD2/gB2-MF59 is a subunit vaccine composed of truncated gD2 and gB2 with M59 adjuvant, an oil-in-water emulsion that includes squalene. This vaccine was evaluated in two randomized, double-blind, placebo-controlled studies. The first included 531 HSV-2 seronegative partners of HSV-2-infected persons, and the second study included 1862 individuals attending a sexually transmitted diseases clinic and at high risk of HSV-2 infection (Table 2) [50]. For the initial 5 months after vaccination, the acquisition rate of HSV-2 infection was 50% lower in vaccine recipients. However, the vaccine was not

successful in preventing infection after 1 year of follow-up and there was no effect on the rate of symptomatic HSV-2 infection, despite inducing neutralizing antibody levels exceeding those induced by natural infection. These results suggest that neutralizing antibodies alone may not be sufficient to protect against genital HSV-2 infection. Pre-existing immunity to HSV-1 did not influence the rate of acquisition of HSV-2 but did increase the proportion of asymptomatic infections.

The gD2-Alum monophosphoryl lipid A (MPL) vaccine is composed of gD2 combined with aluminum hydroxide (alum) and 3-O-deacylated MPL. Two trials were performed and the results reported together [51]. The first trial studied HSV-1 and HSV-2 seronegative partners of persons with a history of genital herpes and showed only 38% vaccine efficacy to prevent genital disease (HSV-1 or HSV-2). The second trial evaluated female partners (regardless of their HSV serostatus) of persons with a history of genital herpes and also showed no significant protection from genital disease (42% efficacy). However, subgroup analyses demonstrated that this gD2 subunit vaccine was protective (73 and 74% efficacy, study 1 and study 2, respectively) against HSV genital disease in HSV-1 and HSV-2 seronegative women, but not in HSV-1 seropositive/HSV-2 seronegative women or in men, regardless of their HSV serostatus.

The results of the initial two gD2 alum/MPL vaccine studies led to the evaluation of this vaccine in a larger vaccine trial (HerpeVac Trial for women) of 8323 HSV-1 and HSV-2 seronegative women [28]. Surprisingly, the vaccine was not effective in preventing genital herpes disease (the primary endpoint) or infection. However, a substudy analysis showed that the vaccine was partially effective in preventing genital infection (35% reduction) and disease (58% reduction) caused by HSV-1. Antibody to HSV correlated with protection from HSV-1 infection. The discordant results of the initial gD2 alum/MPL studies and the Herpevac study are not fully explained; however, the former studies involved women whose 'regular sexual partners' had clinically confirmed genital herpes, while the latter study involved women who were at risk of HSV infection and disease. Interestingly, HSV-1 was a more common cause of genital herpes than HSV-2 in the control group of the Herpevac trial, which emphasizes the importance of a herpes vaccine that can protect against both HSV-1 and HSV-2. The vaccine induced both antibody and CD4⁺ T cells that recognized gD2; thus, the results suggest that these immune responses are insufficient to prevent genital herpes infection and disease.

Attenuated live virus vaccines

Attenuated live virus vaccines should present a broader array of HSV antigens than subunit vaccines, and therefore would be more likely to induce virus-specific CD8⁺ T-cell responses than subunit vaccines. R7020 is an attenuated live HSV-1 vaccine that has a 700 bp deletion in the thymidine kinase gene and a 14 kbp deletion into which are inserted several glycoproteins of HSV-2 [52]. R7020 established latency at a reduced rate in mice, guinea pigs and rabbit models compared with wild-type virus and protected animals against 'severe infections' after challenge with wild-type virus [52]. In clinical studies, the vaccine was poorly immunogenic at the maximum dose tested (10⁵ pfu) [53].

Therapeutic vaccines

Recombinant glycoprotein subunit vaccines

Straus *et al.* studied two glycoprotein subunit vaccines in patients with frequently recurrent genital herpes to test the feasibility of modifying an established HSV infection (Table 3) [54,55]. The primary end point of the trials was the frequency of symptomatic outbreaks of genital herpes. In the first trial, recipients of a recombinant gD2 vaccine with alum experienced significantly fewer virologically confirmed recurrences per month [54]. In the

second trial, in which subjects received a recombinant gD2/gB2 vaccine in MF59 adjuvant, the monthly rate of recurrences was not significantly reduced [55]. However, the duration of new lesion formation, symptoms and time to healing for the first recurrence after vaccination were significantly shortened. The investigators attributed the difference in outcomes of the two studies to the difference in amount of glycoproteins (100 µg gD2 vs 10 µg each of gB2 and gD2) and the different adjuvants (alum vs MF59) used in the vaccines. They concluded that their studies support the concept of a therapeutic vaccine for ameliorating recurrences of HSV-2.

Live virus vaccines

Casonova *et al.* created a live virus vaccine, ICP10ΔPK, in which the protein kinase domain of the large subunit of ribonucleotide reductase was deleted [56]. The virus is impaired in its ability to establish latency in dorsal root ganglia and to reactivate from latency. In animal models, ICP10ΔPK elicited virus-specific CD8⁺ cytotoxic T cells. In cutaneous and vaginal animal models, vaccination with ICP10ΔPK prevented nearly 90% of recurrences [57]. In a small placebo-controlled, double-blind study of 32 patients with frequently recurring HSV genital disease, ICP10ΔPK prevented recurrent HSV-2 genital disease in 37.5% of vaccine recipients from 10 to 180 days after the last vaccine dose ($p = 0.068$) [56]. Vaccinated patients had fewer recurrences (1.58) than placebo recipients (3.13; $p = 0.028$). While these results are promising, recurrences were not documented virologically.

The only replication-defective therapeutic vaccine evaluated in a multicenter, randomized, controlled clinical trial in persons with frequently recurrent genital herpes was the disabled infectious single cycle gH deleted vaccine [58]. The recombinant vaccine virus was propagated in complementing cells that express the *HSV-2 gH gene*. The virus can infect cells but only undergoes a single cycle of replication *in vivo*, since normal human cells do not express gH. The vaccine had no effect on reducing the time to first recurrence of genital herpes after vaccination (the primary end point), clinical disease or genital shedding.

HSV-2 vaccines in early-stage clinical trials

Investigators are applying novel molecular approaches to HSV-2 vaccine development. For example, Cattamanchi *et al.* tested a DNA vaccine consisting of a plasmid expressing gD2 in a Phase I, double-blind, controlled, dose-escalation, safety and immunogenicity trial [59]. gD2-specific cytotoxic T lymphocytes and lymphoproliferation responses were detected in one out of four subjects who received the highest dose of vaccine. Therefore, higher doses of vaccine or adjuvants will likely be required to generate an immune response.

Koelle *et al.* tested a vaccine (HerpV) consisting of recombinant heat shock protein (rhHsc70), an array of HSV-2 peptides (32 synthetic 35 mers predicted to contain HSV-2-specific T-cell epitopes) and a saponin adjuvant [60]. Heat shock proteins have previously been shown to elicit T-cell responses against peptides that they chaperone [61]. The seven participants who had evaluable samples and who received the vaccine had statistically significant CD4⁺ T-cell responses to HSV-2 proteins and most of these subjects also had significant CD8⁺ T-cell responses.

HSV immune evasion genes are important for virus manipulation of the immune system. The smallpox vaccine (vaccinia virus) is lacking a large number of immune evasion genes that contribute to its attenuation. A candidate HSV vaccine, ImmunoVEX^{HSV2} deleted for several immune evasion genes is currently in an early phase clinical trial in the UK [101].

Vaccine approaches for the future

Recombinant glycoprotein subunit vaccines

While gD in alum/MPL failed to protect HSV-1 seronegative women from HSV-2 disease [28], combining other HSV-2 glycoproteins with gD would provide additional immunogens and potentially block the ability of the virus to evade immune responses (Table 4). HSV-2 gC binds the C3b component of complement and inhibits complement-mediated neutralization of the virus [62]; therefore, antibodies to gC2 could block the ability of the virus to evade neutralization by complement. Immunization of mice with combined gC2 and gD2 in CpG and alum increased neutralizing antibody in the presence of complement, and reduced the amount of virus in ganglia after intravaginal challenge compared with animals that received gD2 alone; however, acute disease scores were not significantly better than for animals receiving gD2 alone [63]. Vaccination of guinea pigs with combined gC2 and gD2 in CpG and alum resulted in higher neutralizing antibody titers in the presence of complement, and fewer days of HSV-2 shedding during recurrent infection after challenge compared with animals vaccinated with gD2 alone; however, acute disease, vaginal shedding during acute disease and the frequency of recurrent genital disease after challenge was not reduced compared with animals receiving gD2 alone [63]. These studies suggest that in some settings adding other viral proteins may improve the vaccine's ability to protect against HSV-2 disease.

Inactivated vaccines

While inactivated HSV-2 has not been shown to be protective in humans, these vaccines were not given with newer adjuvants. A recent study compared four vaccines in the guinea pig genital model: empty plasmid vector followed by formalin-inactivated HSV-2 in alum/MPL; vaccination with plasmids that express gD, UL5 and UL30 followed by formalin-inactivated HSV-2 in alum/MPL; vaccination with plasmids that express gD, UL29 and UL52 followed by formalin-inactivated HSV-2 in alum/MPL; or one dose of gD protein in alum/MPL [64]. All four vaccines showed similar levels of protection against acute disease, virus shedding and recurrence rates of genital herpes in guinea pigs. The three vaccines containing inactivated HSV-2 (but not the gD vaccine) significantly reduced the latent virus load compared with control. Thus, an inactivated HSV-2 in an effective adjuvant is another approach that should be considered in addition to replication-defective or live attenuated vaccines.

Replication-defective vaccines

Vaccine HSV-2 *dI5-29* is deleted for two viral genes (UL5 and UL29) that are essential for virus replication and is grown in complementing cells that express these two proteins [65]. HSV-2 *dI5-29* is impaired for latency in mice [66] and guinea pigs [67]. Repeated immunization of mice subcutaneously with HSV-2 *dI5-29* reduced acute virus shedding, vaginal lesions and mortality. HSV-2 *dI5-29* also decreased latent infection after intranasal challenge with wild-type virus HSV-2 [66]. Vaccination of guinea pigs with HSV-2 *dI5-29* reduced acute, recurrent and latent infection as well as shedding after challenge with wild-type virus when compared with unvaccinated animals [67]. Guinea pigs vaccinated with HSV-2 *dI5-29* had higher neutralizing antibody titers and lower rates of vaginal shedding, and lower levels of HSV-2 DNA in the ganglia after challenge than animals vaccinated with HSV-2 gD [68]. Prior infection with HSV-1 did not diminish the effectiveness of HSV-2 *dI5-29* vaccine to reduce acute and recurrent disease after challenge with HSV-2 in guinea pigs when compared with unvaccinated animals. HSV-2 *dI5-29* was >250,000-fold less virulent than wild-type HSV-2 after intracranial inoculation [69].

CJ9-gD is replication-defective HSV-1 vaccine that is engineered to express increased levels of HSV-1 gD very early in infection [70]. The virus has a dominant negative mutation in UL9, which results in inability to replicate both its own viral DNA as well as that of wild-type virus in cells infected with the vaccine strain. Vaccination of mice with CJ9-gD reduced shedding, genital lesions, hind limb paralysis and death after intravaginal challenge with HSV-2 [71]. Vaccination of guinea pigs with CJ9-gD protected the animals from acute genital lesions and hindlimb paralysis, reduced virus shedding, prevented recurrent disease and reduced latent viral DNA after challenge with wild-type HSV-2 [72].

A third promising replication-defective HSV vaccine in development was created by deletion of UL29 and expressing the costimulation molecule, B7, in an attempt to enhance the T-cell immune response in a replication-defective vaccine [73]. Immunization of mice with this vaccine increased the number of IFN- γ -producing T cells and reduced challenge virus replication in the vaginal mucosa, and neurologic and genital disease, and mortality compared with vaccination with replication-defective virus not expressing B7 [74].

Live attenuated vaccines

The only licensed human herpes virus vaccines are the live attenuated varicella vaccine to prevent chickenpox and the zoster vaccine to prevent shingles. Attenuated herpes virus vaccines are used to prevent a number of veterinary herpes viruses including pseudo-rabies virus, Marek's disease and bovine herpesvirus 1. Thus, a live attenuated vaccine may be more likely to be effective than a subunit vaccine; however, safety issues are paramount, especially if the vaccine virus can establish latency.

HSV-2 gE is critical for cell-to-cell spread *in vitro*, including from epithelial cells to axons, from neurons to epithelial cells, and for anterograde transport from neuron cell bodies to axons [75]. An HSV-2 gE deletion mutant was more than 100,000-fold less virulent in mice than wild-type virus after intracerebral inoculation, and caused no death or disease after infection of BALB/c and SCID mice when administered intramuscularly or intravenously [76]. The HSV-2gE deletion mutant had a greater than 100-fold or greater than 100,000-fold higher 50% lethal dose in SCID mice than wild-type virus after intramuscular or intravaginal inoculation, respectively [75]. Two prophylactic immunizations of guinea pigs with the HSV-2 gE mutant resulted in reduced acute vaginal shedding and disease, and reduced recurrent vaginal shedding and lesions compared with control cell lysate after challenge with HSV-2 [76]. Therapeutic immunization of guinea pigs previously infected with HSV-2 reduced the incidence of recurrent genital disease.

HSV ICP0 is an immediate-early protein that functions as an interferon antagonist in both HSV-1 [77] and HSV-2 [78]. Intraocular vaccination of mice with an HSV-2 mutant deleted for the nuclear localization signal of ICP0 (HSV-2 0 Δ NLS) protected the animals from lethal intraocular infection after challenge of the contralateral eye with wild-type HSV-2 [78]. Vaccination of mice with HSV-2 0 Δ NLS was superior to vaccination with HSV-2 gD in alum/MPL in reducing vaginal shedding and mortality after intravaginal challenge with HSV-2 [79]. HSV-2 0 Δ NLS induced higher levels of neutralizing antibody than gD in alum/MPL. However, the majority of severely immunocompromised mice inoculated with HSV-2 0 Δ NLS died from vaccination; thus, the vaccine is likely not sufficiently attenuated for general use [78].

HSV-2 gD binds to several receptors, principally nectin-1, on epithelial cells and neurons, and to herpes virus entry mediator (HVEM) on epithelial cells and lymphocytes. HSV-1 pseudotyped to carry gD mutations showed that amino acid substitutions at amino acids 215, 222 and 223 of gD reduced binding to nectin-1 and impaired nectin-1-mediated entry of virus into cells; however, the mutations did not inhibit binding to HVEM or entry into cells

expressing HVEM [80]. These studies suggested that HSV with gD mutants that impair entry via nectin-1 might be used as vaccine candidates in that they would replicate in epithelial cells in the periphery, but not infect neurons. Based in part on these findings, an HSV-1 candidate vaccine was constructed with a mutation at amino acid 3 of gD; the mutant was impaired for entry into nectin-1 as well as HVEM expressing cells [81]. The mutant was attenuated, and protected mice from acute and recurrent disease and death after challenge with HSV-1 inoculated into the flank. An HSV-2 candidate vaccine with mutations at amino acids 215, 222 and 223 in gD infected human epithelial cells but was severely impaired for infecting neuronal cells [82]. When inoculated into mice, the vaccine was safe and protected against challenge with a lethal dose of wild-type HSV-2 [Wang *et al*, Unpublished Data].

Expert commentary

While the ultimate goal of any vaccine is to induce sterilizing immunity, a more practical goal for an HSV-2 vaccine would be to reduce disease and genital shedding. The only licensed herpes virus vaccine that reduces disease due to primary infection is the varicella vaccine, which diminishes disease associated with varicella, but does not induce sterilizing immunity [83]. In addition, the observation that persons can be infected with more than one strain of HSV-2 implies that sterilizing immunity might not be possible [44]. An HSV-2 vaccine that protects the recipient from primary disease and reduces reactivation and shedding, even without inducing sterilizing immunity, would reduce transmission of virus and potentially alter the epidemiology of HSV-2 infection. Such a vaccine would be a major contribution to public health.

Therapeutic vaccine studies in humans, in which the ‘attack rate’ or frequency of recurrences is high can be performed with far fewer subjects than prophylactic vaccine studies in which the ‘attack rate’ is usually <5%. Thus, there is a preference to perform therapeutic vaccination Phase III trials with HSV-2 vaccines. In our opinion, however, it will be more difficult to achieve success in a therapeutic vaccine trial, as the vaccine would need to induce a more effective immune response than is naturally produced in a person with frequent virus reactivation [84]. Therefore, it is possible that a failed therapeutic vaccine might be fully successful if it is tested as a prophylactic vaccine [58].

In our opinion, efforts should focus primarily on the development of prophylactic vaccines until more is understood about the biology and immunology of reactivation. While numerous virus vaccines are approved to prevent disease associated with primary infection, only the herpes zoster vaccine is licensed to prevent reactivation of a virus infection. Importantly, varicella-zoster virus, which causes shingles, is thought to reactivate only once in most persons, while HSV-2 reactivates on nearly a daily basis [85]. Therefore, while the zoster vaccine is an important precedent for a therapeutic HSV-2 vaccine, the pathophysiology of varicella-zoster virus and HSV-2 reactivation are very different.

Glycoprotein vaccines are likely to be extremely safe and induce high levels of neutralizing antibody; however, they are unlikely to be effective as both a prophylactic and therapeutic vaccine. The observation that an HSV-2 gD vaccine induced antibodies at levels higher than those observed in healthy seropositive persons and did not protect humans from HSV-2 disease [27] suggests that gD alone may be insufficient or that more immunogenic methods are needed to deliver gD. A vaccine that induces both potent antibody and T-cell responses will likely protect against primary disease, as well as reduce reactivation in those who eventually become infected, since cellular immunity is important for control of virus reactivation. The only licensed vaccine that protects against primary infection with a human herpes virus is the live attenuated varicella vaccine, which induces both potent humoral and cellular immunity. This suggests that a live attenuated HSV-2 vaccine would most likely be

successful, if it is sufficiently safe. An alternative and safer approach would be a replication-defective HSV-2 vaccine, assuming it could be sufficiently immunogenic. Such a vaccine could be either a disabled infectious single cycle vaccine that can infect cells and release non-infectious virus or a replication-incompetent vaccine that infects cells, but does not produce virus [86].

The correlates of protection for a prophylactic HSV-2 vaccine are unknown at present. The only licensed viral vaccine that prevents a genital infection is the human papillomavirus vaccine. This vaccine induces antibody titers that are approximately tenfold higher than those seen with natural infection [87]. These high titers might enable the antibodies to reach the mucosal surface as a transudate, which may result in protection from infection [88]. It is important to note, however, that HPV and HSV are very different viruses and that they bind to different cell types. The initial target of HPV during primary infection is the cervicovaginal basement membrane followed by infection of basal epithelial cells [89]. HSV targets mucosal epithelial cells directly. Nonetheless, this suggests that humoral responses to HSV, sufficient to result in high levels of antibody in the genital tract, might result in protection from HSV disease and/or infection.

Five-year view

Since the gD2 Herpevac vaccine did not demonstrate significant efficacy against overall genital herpes disease in HSV-1 and HSV-2 seronegative women, it seems unlikely that a soluble gD vaccine alone will be useful as a vaccine for women and men. Additional HSV glycoproteins, peptide vaccines associated with heat shock proteins or with new adjuvants, or DNA vaccines are likely to be very safe, but it is unclear at present whether they will be sufficiently immunogenic. Safety is a primary concern when vaccinating healthy persons and therefore, in our opinion, a replication-defective vaccine or an inactivated virus vaccine would be appropriate candidates to test in clinical trials. If replication-defective and/or inactivated vaccines are ineffective, a live attenuated vaccine should be tested in clinical trials. Such a vaccine seems most likely to be effective, since it should elicit a broad immune response and a precedent exists with the live attenuated varicella vaccine. However, the safety of a live attenuated HSV vaccine will be of paramount importance.

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Website

101. BioVex Initiates Phase 1 Clinical Trial With Its Genital Herpes Vaccine, ImmunoVEXHSV2. www.medicalnewstoday.com/releases/181424.php

Key issues

- Animal studies suggest that neutralizing antibody responses are important for successful prophylactic vaccination. However, success of a vaccine in animals does not guarantee success in humans, as demonstrated by the recent failure of a recombinant gD2 vaccine to prevent HSV-2 infection or HSV-2 genital disease in humans. Vaccine development should focus on strategies that will elicit a broad immune response that recognizes a diverse set of viral antigens.
- Clinical studies suggest that virus-specific T-cell responses will be necessary for an effective therapeutic vaccine in humans.
- Continued study of the biology of HSV and of immunologic correlates of protection for control of HSV infection are needed for vaccine development. Potent immune responses at the site of infection (genital tract) will be important.
- Replication-defective or live attenuated vaccines that express a broad array of viral antigens and vaccine approaches that use novel molecular strategies, such as complexing multiple viral antigens to heat shock proteins, should be tested in addition to newer approaches using glycoprotein vaccines.
- HSV-2 vaccines that reduce primary disease and shedding are an important goal for an HSV-2 vaccine. While sterilizing immunity would be ideal, the observation that persons can become infected with more than one strain of HSV-2 suggests that this might not be achievable.
- In developed countries, HSV-1 infection is emerging as the most frequent cause of primary genital herpes in young women. Therefore, an effective genital herpes vaccine will need to protect against both HSV-2 and HSV-1.

Table 1

Animal models used for herpes simplex virus-2 vaccine development.

Animal	Advantages	Disadvantages
Mouse	Inexpensive	No spontaneous reactivation
	Immune reagents available	No spontaneous shedding
	Inbred strains, including HLA transgenics, available	Requires medroxyprogesterone for intravaginal infection
Guinea pig	Spontaneous reactivation and shedding	Limited immune reagents
Cotton rat	Spontaneous reactivation and shedding	Limited immune reagents
Owl monkey	Highly susceptible to HSV-2 infection	Animals die with very low titer inoculum

Randomized, double-blind, placebo-controlled human trials of prophylactic recombinant subunit herpes simplex virus-2 vaccines with clinical end points.

Table 2

Composition of vaccine	Phase	Target population	Immunologic response	Outcome	Ref.
Recombinant gD2/gB2 with MF59	III	HSV-2-seronegative members of discordant heterosexual couples or STD clinic enrollees	Strong neutralizing antibody and CD4 T-cell responses	No difference in acquisition rates of HSV-2 infection	[50]
Recombinant gD2 with Alum/MPL	III	Two studies combined: Study 1: HSV-1/HSV-2-seronegative persons whose regular partners have a history of genital herpes Study 2: persons with any HSV serostatus whose regular partners have a history of genital herpes	Induced neutralizing antibody and CD4 T-cell responses	No significant difference in occurrence of genital disease in all HSV-1-and HSV-2-seronegative subjects in study 1 and in HSV-2-seronegative subjects in study 2 (primary end points). Substudy analyses showed vaccine decreased genital disease by 73–74% and HSV-2 infection by ~40% in HSV-1- and HSV-2-seronegative women	[51]
Recombinant gD2 with Alum/MPL – Herpevac trial	III	HSV-1 and HSV-2-seronegative woman	Unsustained increases in anti-gD2 and neutralizing antibody titers	Overall efficacy against genital herpes: 20% (primary end point) No efficacy against HSV-2 infection or disease Efficacy against HSV-1 disease: 58%; efficacy against HSV-1 infection: 35%	[28]

Alum: aluminum hydroxide; MF59: 5% squalene, oil-in-water emulsion; MPL: 3-O-deacylated-monophosphoryl lipid A; STD: Sexually transmitted disease.

Randomized, double-blind, placebo-controlled human trials of therapeutic subunit or live virus herpes simplex virus-2 vaccines with clinical end points.

Table 3

Composition of vaccine	Phase	Target population	Immunologic response	Outcome	Ref.
Recombinant gD2 vaccine with alum	II	Persons with symptomatic genital HSV-2 of at least 1-year duration with 4–14 outbreaks/year	Neutralizing antibody titers boosted fourfold; gD2-specific titers boosted sevenfold	Reduced rate of virologically confirmed monthly recurrences ($p = 0.019$); lower median number of virologically and/or clinically confirmed recurrences per patient during the study year ($p = 0.025$)	[54]
Recombinant gD2/gB2 vaccine with MF59	II	Persons with symptomatic genital HSV-2 of at least 1-year duration with 4–14 outbreaks/year; western blot positive for HSV-2 antibodies	Boosted glycoprotein-specific and neutralizing antibodies for the duration of the study	Monthly rate of recurrence was similar for vaccinated and placebo control (primary end point); duration and severity of first confirmed study recurrence significantly reduced	[55]
Replication competent virus ICP10ΔPK (mutation in <i>UL39</i> gene)	I/II	Persons with at least five documented recurrences of genital herpes	Not reported	Genital HSV-2 disease prevented in 37.5% of vaccine recipients and in no placebo participants ($p = 0.068$ for total episode comparison); vaccinated subjects had fewer recurrences ($p = 0.028$); no virologic assessment performed	[56]
Replication defective-disabled infectious single cycle mutant (gH deletion)	III	Persons with 6 recurrences per year	No immunologic benefit	No effect on the time to first recurrence of genital herpes (primary end point); no difference in time to lesion healing or mean number of recurrences	[58]

Alum: Aluminum hydroxide; MF59: 5% squalene, oil-in-water emulsion.

Table 4

Selected vaccines in preclinical studies.

Vaccine	Property	Results in animals	Ref.
Glycoprotein gC + gD	Blocks HSV-2 immune evasion from neutralization by complement	Reduces latency and vaginal shedding in mice and guinea pigs, respectively	[63]
Inactivated virus in alum/MPL and HSV-2 plasmids	Induces neutralizing antibody	Reduces latency and recurrent disease in guinea pigs	[64]
Replication-defective (dl-5-29)	Induces neutralizing antibody and CD8 T-cell responses	Reduces latency and mortality in mice; reduces latency, acute and recurrent disease in guinea pigs	[65–68]
Replication-defective, CJ9-gD	Overexpresses HSV-1 gD; gD expressed very early in infection	Reduces shedding, genital lesions, hindlimb paralysis and death after intravaginal challenge of mice with HSV-2 and reduces shedding and recurrences in guinea pigs	[70–72]
Replication-defective B7-expressing, 5B-86	Increased IFN- γ -expressing T cells	Reduces viral replication in vaginal mucosa, genital and neurologic disease and mortality	[73,74]
<i>Live attenuated vaccines</i>			
HSV-2 gE-null mutant	Defective anterograde transport and cell-to-cell spread	Reduces acute, recurrent disease and shedding in guinea pigs; reduces recurrent disease in previously infected guinea pigs	[75,76]
HSV-2 Δ NLS	Reduces activity of ICP0, an interferon antagonist	Reduces vaginal shedding and mortality after intravaginal challenge with HSV-2 compared with gD in alum/MPL	[77,79]
HSV-2 with mutations in gD	Little or no infection of cells expressing nectin-1 in the absence of herpes virus entry mediator – an HSV receptor	Protects mice from lethal infection	[82] [Wang <i>et al.</i> , Unpublished Data]

Alum: Aluminum hydroxide; MPL: 3-O-deacylated-monophosphoryl lipid A.