HSV-2: in pursuit of a vaccine

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Herpes simplex virus type 2 (HSV-2) is one of the most prevalent sexually transmitted infections worldwide. In addition to recurrent genital ulcers, HSV-2 causes neonatal herpes, and it is associated with a 3-fold increased risk for HIV acquisition. Although many HSV-2 vaccines have been studied in animal models, few have reached clinical trials, and those that have been tested in humans were not consistently effective. Here, we review HSV-2 pathogenesis, with a focus on novel understanding of mucosal immunobiology of HSV-2, and vaccine efforts to date, in an attempt to stimulate thinking about future directions for development of effective prophylactic and therapeutic HSV-2 vaccines.

Introduction

Herpes simplex virus type 2 (HSV-2) is a sexually transmitted pathogen that infects more than 500 million people worldwide and causes an estimated 23 million new infections each year (1). HSV-2 seroprevalence ranges from 16% among 14–49 year olds in the United States (2) to greater than 80% in some areas of sub-Saharan Africa (3); seroprevalence in women is up to twice as high as men, and increases with age (2, 4). Although HSV-2 is the leading cause of genital ulcer disease worldwide (5, 6), most people are not aware of the infection (7), and may transmit the virus during periods of subclinical shedding (8, 9). In contrast to other sexually transmitted infections (STIs) that may be concentrated among "core groups," such as gonorrhea (10), HSV-2 is widespread even among people with low or moderate levels of sexual activity. For instance, 18.8% of American women with 2–4 lifetime sexual partners are HSV-2 seropositive (2).

Although incident genital herpes is increasingly caused by HSV type 1 (HSV-1; ref. 11), and HSV-1 may also cause significant eye and brain disease, almost all HSV vaccine candidates reaching clinical trials have targeted HSV-2. As HSV-1 and HSV-2 have similar pathogenesis and host interactions, many of the concepts for development of an effective vaccine are likely relevant to both viruses. In addition, infection with HSV-2 provides partial protection against HSV-1 (12), although the reverse does not appear to be true (13), and thus there is potential for generation of cross-reactive immunity (14). The possibility that an HSV-2 vaccine may provide protection against HSV-1 increases its potential value and may shift the optimal time for immunization to early childhood, instead of the more problematic adolescent vaccination series (15).

Transmission of HSV from mother to infant during birth is the most serious complication of genital herpes, and women who acquire HSV during pregnancy are at the highest risk of transmitting the infection (16). Neonatal herpes often results in long-term neurologic sequelae or mortality (17). The estimated incidence of neonatal herpes varies widely, from 4 to 31 in 100,000 live births (12, 18). Although neonatal herpes is too rare to be used as an endpoint in a clinical vaccine trial, prevention of HSV acquisition during pregnancy is an important goal of developing an effective HSV vaccine.

The risk of HIV-1 acquisition is 3-fold higher among HSV-2–seropositive persons (19); in populations with 80% seroprevalence, nearly 50% of HIV infections are attributable to prevalent HSV-2 (20). The mechanism of increased risk of HIV acquisition includes influx of HIV target cells (21) in response to HSV-2 replication in the genital mucosa. Thus, decreasing either HSV-2 susceptibility or reactivation through prophylactic vaccination could lead to a marked decrease in HIV incidence in sub-Saharan Africa (22).

Prevention strategies for sexual transmission of HSV-2 include condom use (23), disclosure of serostatus (24), and suppressive antiviral therapy (23). However, these methods are imperfect, as each reduces transmission by only approximately 50% (25, 26). Moreover, antiviral therapy does not abrogate the increased risk of HIV acquisition (27, 28) or transmission (29) in HSV-2–seropositive persons. The currently available strategies are useful for individual patients, but they are unlikely to be of public health benefit. A prophylactic vaccine would be valuable from both the patient and public health standpoint, if it were able to meet or exceed the efficacy of currently available preventive therapies.

New insights into HSV pathogenesis: frequent and dynamic reactivation

HSV infects epithelial cells at skin and mucosal surfaces during primary infection, then travels via retrograde transport along nerve axons to the dorsal root ganglia (DRG), where latency is established (30). While epithelial cells are destroyed during lytic HSV replication, neuronal cells are not destroyed and provide a reservoir for latent virus. During reactivation, the virus travels from the DRG back to the skin and causes detection of virus from epithelial surfaces (known as viral shedding). Viral reactivation may be asymptomatic or may be associated with prodrome (tingling or burning), nonspecific symptoms or lesions, or a classic genital ulcer.

Studies that measure the frequency of viral shedding and the quantity of virus detected from the genital tract have provided insight into the natural history and pathogenesis of HSV-2 infection. HSV was previously thought to be in the latent state most of the time during chronic infection, with rare clinically evident

reactivation episodes, conceptually similar to varicella zoster virus (VZV). However, shedding studies using anogenital swabs collected by HSV-2–seropositive persons once or multiple times daily for viral culture or for quantitative real-time PCR have revealed that HSV and the host are in constant conflict, with frequent HSV reactivation and rapid clearance (31). This implies host responses that likely involve cell-intrinsic and -extrinsic mechanisms of innate as well as acquired immunity. In studies in which HSV-2–infected persons collect daily genital swabs, HSV is detected on a median of 12%–28% of days (32) and is found on 10% of days even among persons with asymptomatic HSV-2 infection (33). Many of these shedding episodes occur in the absence of lesions or symptoms (known as subclinical shedding; ref. 34). Mathematical models suggest that multiple, short, overlapping shedding episodes best simulate the observed shedding patterns (35) and that a nearly constant low quantity of HSV is likely to be released from sensory DRG into the genital tract (36). Recent studies using intensive sampling (every 6 hours) of the genital and oral mucosa have produced results consistent with these models, demonstrating that most HSV detection episodes are short (median 13 hours), subclinical, and rapidly cleared (31). In addition, studies using detailed genital mapping to isolate shedding episodes have demonstrated that simultaneous, bilateral widespread genital shedding is detected frequently (37). HSV lesions are associated with persistent infiltrate of CD4+ and CD8+ T cells in the mucosa (21, 38) and at nerve endings (38) that may contribute to a chronic inflammatory state in genital skin and mucosa. In support of this hypothesis, histopathologic studies of foreskin in HIV-seronegative men after adult circumcision have shown a higher concentration of CD4+ and CD8+ T cells in HSV-2–seropositive compared with HSV-2–seronegative men (39). Thus, even persons with established infection and a functional immune system can experience both subclinical genital HSV shedding and lesional recurrences, which suggests that the virus can evade even mature host immune responses. These findings indicate that chronic HSV infection involves a dynamic equilibrium between ganglionic and mucosal compartments.

HSV-2 transmission dynamics

HSV-2 infection rates in heavily exposed populations, such as commercial sex workers, are nearly 100%, suggestive of near-universal susceptibility (40). Studies in HSV-2–discordant couples have shown that most HSV-2 transmissions occur during periods of subclinical shedding in the source partner (8). The relationship between the inoculum dose, the presence of local abrasions or other epithelial variables, and the likelihood of successful transmission has not been established in humans. An increase in the inoculum dose required to produce disease or establish ganglionic latency might result from a vaccine, as has been shown with a live attenuated candidate vaccine in guinea pigs (41), providing partial protection from infection or disease.

HSV-2 is transmitted between sexual partners fairly quickly in a new sexual relationship. In a cohort of 199 patients with laboratory-documented primary HSV-2 infection and a clearly defined transmitting partner, the median number of sexual acts between the couple at the time of HSV-2 acquisition was 40 (24). Obversely, our group has identified some HSV-2–seronegative persons in long-term HSV-2–discordant sexual relationships who have T cell responses to HSV-2 (42), which suggests that not all exposures result in infection. Preliminary data suggest that these T cell responses may be weighted toward certain HSV-2 proteins (42). If confirmed and extended

using the whole HSV-2 proteome, this finding could indicate preferred compositions for future subunit vaccines.

Experts have long considered infection by multiple HSV-2 strains in a given individual a rare phenomenon (43, 44), raising the potential for inducing sterilizing immunity. However, recent studies using more sensitive PCR techniques and newly identified variable regions in HSV-2 have found multiple-strain genital infection in 15% of healthy adults infected with genital HSV-1 and HSV-2 (45, 46). In a small cohort of HIV/HSV-2 coinfected persons, all 11 harbored more than one HSV-2 strain; however, the relative contributions of high sexual exposure versus immunosuppression to this observation has not been defined. Sterilizing immunity with other human herpesvirus infections is variable: wild-type infection with VZV protects against reinfection, whereas multiple strains of CMV are detected in more than 90% of CMV-infected women (47). A more detailed analysis of the prevalence of multistrain HSV-2 infection is now possible because of increased knowledge of viral SNPs (48, 49) and can be used to establish how well naturally occurring HSV-2 infection protects against infection with a second strain in humans. These data will discern whether immunity better than, or perhaps fundamentally different from, that provoked by wild-type infection will be a necessary component of a successful vaccine.

The immune response to HSV-2

HSV-2 induces an immune response that eventually contains the virus, as suggested by the temporal correlation of the infiltration of antigen-specific CD4+ and CD8+ T cells at the site of a lesion and clearance of virus (50). The importance of the host immune response is demonstrated by the severe, prolonged ulcerations that can occur in patients with AIDS (51) or after solid organ (52) or stem cell transplantation (53). The host and viral determinants of the heterogeneous clinical and virological manifestations of genital HSV-2 are poorly understood. In our opinion, identification of those components of the host immune system that result in containment of viral reactivation from neurons, and viral clearance from the mucosa, will be essential for development of a successful HSV-2 vaccine. This is most likely to be gained by detailed immunologic and genetic studies of persons with well-defined HSV-2 severity.

HSV stimulates the innate immune system to produce IFN-α, through interactions with plasmacytoid DCs (pDCs; ref. 54) and other cells, via TLRs, including TLR9 (55), TLR2 (56), and TLR3 (57). Cytoplasmic DNA sensors, such as stimulator of IFN genes (STING), mediate production of IFN in response to HSV infection, as demonstrated in cell culture and in knockout mouse models (58). Interestingly, most host mutations associated with fatal HSV in childhood occur at loci classically associated with innate immunity, such as TLR3 (57) and UNC93B1 (59), although some mutations associated with severe HSV are in genes involved in both innate and acquired immune responses (e.g., STAT1), reflecting a complex codependence and interaction between these pathways (60). Although innate immune system agonists can lead to profound, local HSV resistance (61, 62), these interventions are outside the realm of classic vaccinology. Single nucleotide polymorphisms in TLR2 are associated with more frequent HSV shedding and genital lesions (63), which suggests that there may be a host genetic contribution to the wide heterogeneity of clinical HSV presentation. Curiously, IFN-α and the characteristic IFN type I gene signature are not present in biopsies of genital HSV-2 lesions (64), which indicates that despite HSV's powerful ability to trigger IFN-α in pDCs, there may be a defect in local type I

IFN responses in situ. HSV-1 has also evolved mechanisms to downregulate IFN expression, for example, via IRF3 degradation mediated by immediate early infected cell protein 0 (ICP0), which prevents accumulation of IFN-β (65). In addition, expression of ICP0 alone can prevent TLR2-dependent NF-κB activation in cell culture, which suggests that the virus downregulates the immune response at several points in the inflammatory pathway (66).

CD4+ T cells are an important source of IFN-γ, have cytotoxic effector activity for HSV-infected cells, and localize to HSV-2 genital lesions (50). CD4+ T cell depletion is statistically associated with increased HSV-2 shedding in HIV-1 infection (67), albeit in the context of a multifaceted immune deficiency, including a profound reduction in circulating pDCs (68). In mice, CD4+ T cells play key protective roles after whole virus vaccination, as shown by depletion studies (69, 70). The presence of CD8+ T cells is associated with HSV clearance from the peripheral nervous system (71), and ganglionic HSV-specific T cells appear to be important in maintaining HSV-1 latency in the mouse model of infection (72). HSV-specific CD8+ T cells are chronically localized to HSV-infected DRG in humans and appear to be activated (73). Mathematical models predict that the duration and severity of HSV shedding episodes is strongly associated with a low density of CD8+ T cells in the genital mucosa (74). In studies using a subset of the predicted HSV proteome, HSV-2–seropositive persons generate a broad CD8+ T cell response, reacting to a median of 11 epitopes (75). These cells typically target immediate-early or early HSV genes and produce IFN-γ, but may be capable of proliferation, IL-2 and TNF-α production, and cytolytic responses (e.g., increased expression of CD107a). No acquired cellular immunity correlates of control of HSV reactivation or shedding have been elucidated in humans.

Neutralizing antibodies to viral envelope glycoproteins, which are required for viral entry into cells, develop in response to HSV infection (76) and may provide some type-common protection against HSV acquisition (77) or reduce the severity of HSV infection (78, 79). As a result, glycoprotein B (gB) and gD subunit vaccines have been pursued in human clinical trials. Physiologically relevant host receptors have been defined (80), and crystallographic structures of HSV proteins with their ligands and dynamic structural changes during viral entry are newly available (81–83). These data may inform the design of a newer generation of glycoprotein-based vaccines designed to elicit optimal neutralizing antibodies, inspired by the success of HPV vaccines, which appear to work at least in part by eliciting higher titers of neutralizing antibodies than those induced by natural infection.

Immune evasion

Alphaherpesviruses, such as HSV-2, have coevolved with primate hosts for millions of years and have developed many strategies to evade the host immune response. The latency associated transcript blocks apoptosis and may allow for survival of neurons during latent infection (84), whereas other viral genes block apoptosis induced by cytolytic immune effector cells (85). Activated HSV-specific CD8+ T cells are found surrounding trigeminal DRG in HSV-1–infected persons, but there is no evidence that these cells kill the neurons, despite the expression of cytolytic proteins such as granzyme B (73). Several HSV proteins, such as the ubiquitin ligase ICP0, interact with the IFN pathway. Contact of T cells with HSV-infected cells inhibits and alters T cell signaling via intracellular phosphorylation cascades (86), and ICP47 blocks CD8+ T cell responses by interactions with the transporter associated with antigen processing (TAP), which moves peptides to MHC class I (87). With the knowledge of specific HSV immune evasion mechanisms, it is possible to create replication-competent or -incompetent virus-based vaccines modified to disable inhibitors of innate and acquired immunity. Examples include deletion of UL41 (which encodes a protein with complex properties, including DC downregulation; ref. 88) from candidate vaccine strains (89) and deletion of multiple immune evasion genes from Immunovex^{HSV-2}, a live attenuated strain currently in phase I clinical trials (90).

Thus, although the components of protective immunity remain unknown, the tools to perform studies to correlate immunity with phenotype are now largely in place: immunocompetent subjects can be phenotyped for shedding frequency, the viral proteome is manageably sized for comprehensive immune studies, and specimens can include infected tissues as well as blood.

Animal models of HSV vaccination

The mouse and guinea pig models of HSV-2 infection have provided important insight into the immunobiology of genital herpes. The mouse model is limited because HSV does not spontaneously reactivate from mice DRG and therefore does not simulate infection in the human host. Some viral immune evasion mechanisms are also less active in mice. Although pathogenesis insights into tissue-resident HSV-specific T cells made in mice appear applicable to human infection (91, 92), most vaccine studies in mice use lethality as an endpoint and, due to space constraints, will not be reviewed here. The guinea pig does reactivate HSV spontaneously for a limited period of time after vaginal infection (93), but few reagents are available to parse out the components of an effective immune response in this model. As an alternative, the cotton rat model, in which HSV reactivation does occur, should be further exploited (94).

Many prophylactic vaccines have been tested in the guinea pig model, and most have been successful in preventing clinically apparent infection (Supplemental Table 1; supplemental material available online with this article; doi:10.1172/JCI57148DS1). In addition, several therapeutic vaccines prevent recurrences in this model. Approaches have included recombinant glycoprotein subunit vaccines, DNA-based vaccines using plasmids encoding glycoproteins, live virus vector vaccines with glycoprotein inserts, live-attenuated HSV-2, and replication-incompetent virus. These vaccines resulted in decreased severity of primary disease, rate of recurrent infection, and quantity of virus shed during primary infection and recurrent activation.

Studies in the guinea pig have shown the quantity of HSV that establishes latency in the DRG correlates with frequency of clinical recurrence (95), which suggests that partial protection could be a valuable endpoint goal in vaccine design. Several candidate vaccines appear to reduce the quantity of HSV in the guinea pig DRG. However, once DRG are infected, shedding still occurs at a rate similar to that of nonimmunized animals (96, 97). These data suggest that once neuronal infection in the DRG is established, the pathobiology of frequent reactivation may not be affected by a therapeutic vaccine, although clinical recurrences and quantity of virus shed may be diminished. In parallel, long-term studies of HSV-2–infected persons show that most, if not all, reactivate HSV-2, albeit with variable frequency (98).

In the HIV vaccine field, a new pipeline for preclinical testing of T cell–based vaccines in nonhuman primates has been proposed (99), which requires efficacy for a clinically relevant, predefined virologic endpoint, followed by examination of immunogenicity

Table 1
Randomized, double-blind, placebo-controlled trials of prophylactic HSV-2 vaccines tested in humans

Vaccine	Population	Phase	Adjuvant	Vaccine schedule	Outcome	Result and comment
Purified glycoprotein						
Glycoproteins (gB, gC, gG, gE, gD) purified from lysed chick embryo fibroblasts infected with HSV-2 (124)	HSV-2 seronegative persons (N = 22)	I	None	9, 28, 140 days	Tolerance and immunogenicity	Safe (124).
Glycoproteins (gB, gC, gG, gE, gD) purified from lysed chick embryo fibroblasts infected with HSV-2 (124)	HSV-2 seronegative, discordant couples (N = 161)	II	None	0, 4, 22 weeks	Time to HSV-2 acquisition and immunogenicity	Time to acquisition was the same in vaccine and placebo recipients. Vaccine had poor immunogenicity for gB2 and gG2 (125).
Recombinant glycoprotein subunit						
Recombinant gB2 and gG2 (Chiron) (100, 101)	HSV-2–seronegative (N = 137)	I/II	MF59	0, 1, 6 months	Neutralizing HSV-2 antibody and proliferative response, safety	Safe. Levels of neutralizing antibody similar to or higher than naturally occurring infection, gD2 and gB2 T cell responses produced (101).
Recombinant gB2 and gG2 (Chiron) (100, 101)	HSV-2–seronegative; discordant couples (N = 531) and STD clinic attendees (N = 1,862)	III	MF59	0, 1, 6 months	Time to HSV-2 acquisition	Initial 50% lower rate of HSV-2 acquisition in vaccine recipients; however, overall vaccine efficacy 9% (95% CI, −29% to 36%). No difference in rate of symptomatic HSV-2 acquisition (100).
Recombinant gD2 (GlaxoSmithKline) (102)	No evidence of genital herpes disease (N = 7,460)	II	Alum plus MPL (AS04)	0, 1, 6 months	Safety and immunogenicity	Safe. Vaccine elicited higher titers of gD2 antibody than natural infection (102).
Recombinant gD2 (GlaxoSmithKline) (102)	Discordant couples; 2 studies, HSV-1– and HSV-2–negative (N = 847), HSV-2–negative (N = 1,867)	III	Alum plus MPL (AS04)	0, 1, 6 months	Genital herpes disease	Overall vaccine efficacy 38% (95% CI, −18% to 68%); HSV-1– and HSV-2–seronegative women, vaccine efficacy 73%–74% (P = 0.01–0.02). Not effective in men or in HSV-1–seropositive women (103).
Recombinant gD2 (GlaxoSmithKline) (102)	HSV-1– and HSV-2–negative women (N = 8,323)[A]	III	Alum plus MPL (AS04)	0, 1, 6 months	Time to genital herpes disease	No difference in rate of genital herpes; estimated vaccine efficacy 20% (126).
Other						
DNA plasmid expressing gD2, needle-free injection (127)	HSV-2–seronegative (N = 62)	I, dose-escalating	None	0, 4, 8, 24 weeks	Safety and immunogenicity	Safe. gD2 T cell responses (CTL and lymphoproliferation) in 1 of 4 participants (highest-dose group only).
Recombinant human HSP-70/ gB HLA-restricted epitope (128)	HSV-1– and HSV-2–seronegative, HLA A*0201 (N = 19)	I, dose-escalating	Human HSP-70	0, 2, 4 weeks	Safety and immunogenicity	Safe. No change in ELISPOT response to gB epitope from baseline; no change with increasing dose.

MPL, 3′-O-deacylated-monophosphoryl lipid A; HSP-70, heat shock protein–70. [A]Herpevac trial.
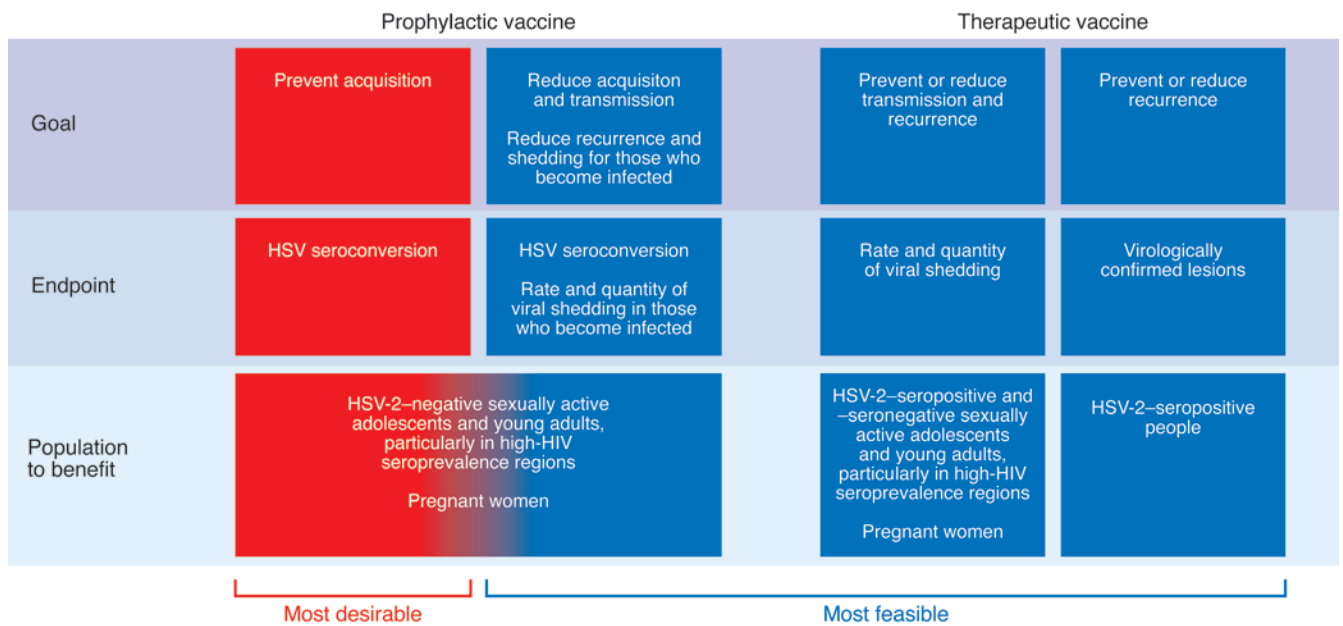
Figure 1
HSV-2 vaccine development strategies.

prior to use in human volunteers. HSV vaccine researchers should also consider virologic efficacy criteria when evaluating prophylactic and therapeutic vaccines, such as reduction or elimination of mucosal shedding or reduction of the establishment of sacral DRG latency, in addition to immunogenicity, in animal models prior to initiating human trials.

Human clinical trials

Prophylactic vaccines. Testing prophylactic HSV vaccines for efficacy requires prospective follow-up of persons at risk for HSV-2 acquisition. Even in high-risk settings, the seroconversion rate is likely to be only approximately 5% per year, making such trials a costly endeavor, similar in scope to HIV vaccine efficacy trials. Substantial efforts have been invested into glycoprotein subunits as vaccine targets, with more than 20,000 human volunteers studied in clinical trials (Table 1). A recombinant gB2 and gD2 subunit vaccine formulated with the adjuvant MF59 was safe and induced strong neutralizing antibody responses and as well as CD4+ T cell responses (100, 101). However, this vaccine was not successful at preventing HSV-2 infection in HSV-2–negative members of discordant heterosexual couples or STD clinic enrollees (100).

Two parallel studies showed that a recombinant gD2 subunit vaccine with an alum/monophospholipid A adjuvant (AS04) was also safe and induced both neutralizing antibody and CD4+ immune responses (102). The first trials randomized HSV-2–seronegative persons in a stable HSV-2–discordant sexual relationship, in which the source partner had clinically evident genital herpes, to receive vaccine or placebo. Although the vaccine was not efficacious in men or HSV-1–seropositive women, the vaccine reduced HSV-2 disease by 70% and HSV-2 infection by 40% in a subgroup analysis of HSV-1– and HSV-2–seronegative women (103). To further evaluate this vaccine, more than 8,000 sexually active HSV-1– and HSV-2–seronegative women were enrolled in the Herpevac trial, a collaborative study sponsored by the NIH and Glaxo-

SmithKline. Preliminary results of the Herpevac trial showed no efficacy against HSV-2 disease or infection (104). These negative trials, while disappointing, have provided insights into the optimal study population, design, and endpoints for future investigations. The fact that different results were found in the two trials raises questions about whether risks of transmission and/or behavior in discordant couples in a stable relationship may be fundamentally different from those with multiple partners (104). Future trials should enroll members of both discordant monogamous couples as well as individuals with multiple partners to further evaluate this. Endpoints of interest in a prophylactic vaccine study include both acquisition of HSV-2 infection, to measure the ability of the vaccine to induce sterilizing immunity, and frequency of viral shedding. Assessment of viral shedding is more precise than identification and attribution of genital signs and symptoms, and lack of viral shedding will undoubtedly lead to lack of clinical manifestations of genital herpes. In addition, viral shedding frequency in those who are infected is also of interest to evaluate a potential effect on dynamics of transmission (Figure 1 and ref. 105).

Therapeutic vaccines. Therapeutic HSV-2 vaccines have been pursued both to improve the clinical course in individual patients and to potentially decrease HSV shedding, and hence transmission, for a public health benefit (Table 2). Most have been safe and have induced a measurable immune response. Although the first study of a recombinant gD2 vaccine adjuvanted with alum reduced the rate of virologically confirmed recurrences (106), later studies of glycoprotein vaccines failed to replicate that effect (107). Unlike the prophylactic vaccine studies, shedding data from our group indicate that the pivotal therapeutic vaccine studies can be performed efficiently with fewer than 100 individuals if viral shedding is used as an endpoint (33), whereas prophylactic vaccination studies require a much larger sample size. Viral shedding rate and quantity of virus shed could serve as useful surrogate endpoints for recurrence rate and transmission likelihood in studies of therapeutic vaccines as well.

Table 2
Randomized, double-blind, placebo-controlled trials of therapeutic HSV-2 vaccines tested in humans

Vaccine	Population	Phase	Adjuvant	Dosing	Outcome	Result and comment
Inactivated virion or killed						
Subunit HSV-1 vaccine (129)	"Frequent herpes" (N = 42)	II/III		Repeated doses	Frequency of recurrences	No difference in recurrence rate compared with placebo.
Inactivated virion–derived HSV-1 subunit vaccine (130)[A]	HSV-2–seropositive with >1 year infection and ≥6 recurrences per year (N = 316)	II/III	None	0, 1, 2 months	Frequency and severity of recurrences	Safe. Vaccine produced neutralizing antibody and cellular immune responses. No significant decrease in recurrence frequency; however, recurrence severity decreased.
Recombinant glycoprotein						
Recombinant gD2 (106)	HSV-2–seropositive with 4–14 outbreaks per year (N = 98)	II	Alum	0, 2 months	Rate of recurrences at 1 year, immunogenicity	Vaccine increased gD2 neutralizing antibody titers. Lower median number of recurrences per year (4 vs. 6 in placebo, $P = 0.039$). No difference in time to first genital recurrence.
Recombinant gB2/gD2 (107)	HSV-2–seropositive with 4–14 outbreaks per year (N = 202)	II	MF59	0, 2, 12, 14 months	Rate and number of first recurrence for first 8 months of study, severity and duration of first recurrence	Vaccine produced increased levels of glycoprotein-specific and neutralizing antibodies. No significant difference in recurrence rate. Duration of first recurrence was slightly reduced in vaccine recipients.
Other						
Recombinant human HSP-70/glycoprotein	HSV-2–infected, HLA A*0201	I	Human HSP-70	0, 2, 4 weeks	Safety and immunogenicity	Safe. No change in ELISPOT response to gB epitope from baseline.
Live virus, attenuated or replication incompetent						
Live, attenuated growth-compromised HSV-2, deletion in UL39 (110)[B]	HSV-2–seropositive with 5 recurrences in previous year (N =32)	I/II	None	7, 17, 28 days after lesion	Proportion of persons with and number of self-reported recurrences 6 months after vaccination	Recurrences completely prevented in 37.5% of vaccinated persons vs. 0% of placebo recipients. Decreased number of self-reported genital herpes recurrences in vaccine arm (1.6 vs. 3.1 in placebo arm). Study limited by lack of virologic confirmation of recurrences.
DISC HSV-2, deletion in gH gene (111)[C]	Healthy persons with ≥6 genital herpes recurrences in previous year (N =485)	III	None	0, 8; 0, 4, 8; or 0, 2, 4, 8 weeks	Time to first genital herpes recurrence over 1 year	Median time to first recurrence and time to lesion healing same in all groups; similar genital HSV shedding rates in participants who received vaccine and placebo.

[A]Skinner vaccine. [B]ICP10ΔPK deletion. [C]Replication incompetent. HSP-70, heat shock protein–70; DISC, disabled infectious single cycle.

Live-virus vaccines: lessons from VZV. In contrast to HSV, there is an effective vaccine against VZV, the other human pathogen in the alphaherpesvirus family. This live-attenuated virus vaccine prevents primary infection and VZV reactivation (zoster; ref. 108), making it the first example of a successful therapeutic vaccine. Whereas antibody titers to VZV do not correlate with risk of reactivation, a boosted cell-mediated immune response is associated with a decreased risk of zoster (109). These lessons should be applied to therapeutic HSV vaccine development in order to ensure that vaccine candidates induce strong cell-mediated immune responses.

Only one replication-competent HSV vaccine has been tested as a therapeutic entity in humans. This virus had a mutation in the UL39 gene and was associated with a decreased number of self-reported genital herpes recurrences compared with placebo (110); unfortunately, there was no virologic assessment of recurrences (Table 2). A disabled infectious single cycle (DISC) mutant with a gH deletion was tested in persons with symptomatic HSV-2, but neither shedding rates nor recurrences rates were affected (111). In this study, a limited titer of virus was used; therefore, the potential for this type of candidate vaccine has not yet been fully explored. However, several novel genetically engineered replication-incompetent vaccine candidates are in the pipeline. For example, the HSV-1 construct CJ9-gD, which is engineered to overexpress gD1 and has a dominant-negative mutation in the origin of binding protein UL9 (which inhibits viral replication), was able to protect guinea pigs from HSV-2 intravaginal challenge, with marked reduction in viral titer and lesion formation as well as amount of challenge virus establishing latency in immunized animals (112). Another pair of promising replication-incompetent vaccine constructs, dl5-29 and dl5-29-41L, with deletions of essential early genes (and, in the -41 virus, the gene encoding the vhs protein), has also been effective at preventing primary infection and decreasing the titer of challenge virus establishing latency in the guinea pig model (89, 113). Replication-competent strains of HSV-1 with a deletion in the gE gene, which is required for neuronal spread and anterograde transport of viral components (114), or of HSV-2 with a deletion in both copies of ICP0, which has multiple virulence and immune evasion functions (115), are other innovative vaccine constructs that will require additional testing in animal models prior to moving into clinical trials.

Goals of vaccination

One of the key concerns in HSV vaccine development is whether sterilizing immunity will be feasible (116). Ideally, an HSV vaccine will be better than nature through a combination of the following mechanisms: (a) inducing sterilizing immunity in the genital tract, (b) preventing initial infection of the DRG, (c) stimulating an immune response that does not allow frequent reactivation of HSV-2 from sacral DRG, or (d) leading to high concentrations of effector cells in genital epithelia to minimize replication after virus is delivered from neurons (92). Mouse models typically require vaginal inoculation with live HSV to produce sterilizing immunity (117), and in the guinea pig model, most vaccines tested have not prevented infection of the DRG. HPV vaccines, which use virus-like particles, induce higher levels of neutralizing antibodies and memory B cells than seen in natural infection (118) and prevent genital infection with high efficacy (119), which demonstrates that vaccines that induce more potent immunity than natural infection are possible.

An alternative vaccination goal is to alter the natural history of HSV infection, by decreasing the quantity of virus establishing latency in neurons and minimizing the quantity of virus shed from genital surfaces (Figure 1). Mathematical modeling analyses have suggested that even vaccines with relatively low efficacy at preventing primary infection could have a substantial effect on the epidemic by decreasing shedding and reducing viral transmission (105). Such a vaccine would have the highest impact in high-prevalence populations (120); it is estimated that a vaccine that only marginally decreases HSV-2 susceptibility, but reduces shedding frequency among vaccinated persons who become infected by 75%, could reduce HSV-2 incidence by 30% over a 10-year period (121). Therapeutic vaccines that reduce shedding could also have a powerful effect on the HSV-2 epidemic (122).

Future directions

Over the last two decades, the field of HSV pathogenesis has progressed rapidly. There is a better understanding of the function of many HSV genes and proteins, as well as the host immune response. Importantly, we have a better appreciation for how frequently HSV reactivates from latency and is shed on mucosal surfaces, allowing for transmission to a new host. These findings point to viral shedding as an appropriate endpoint for proof-of-concept studies. Ideally, a prophylactic vaccine would result in sterilizing immunity, which would not allow HSV to establish latency in the DRG. However, achievement of such a difficult goal will likely require additional research to understand both effective innate and acquired immunity, such that the immune response generated by the vaccine is more potent than that occurring in natural infection.

If the goal of a vaccine is to modify natural history in those already infected, or to alter the course of the infection in those with newly acquired HSV-2, we believe that vaccines should focus on changes in HSV shedding frequency and quantity in those who become infected, in parallel to HIV vaccine studies aimed at modifying the viral setpoint (123). Additional research should be devoted to establishing whether a virologic threshold for transmission exists, and toward a quantitative assessment of the prevalence of multistrain infections. Modeling studies have provided us with the ability to see how imperfect vaccines could work on a population basis; we should take advantage of such studies to have a lively debate about what type of vaccine would be attainable and effective from a personal and public health perspective. A final challenge will be having buy-in from pharmaceutical companies and regulatory bodies to pursue the potentially risky strategy of investing in a vaccine that decreases shedding rather than prevents infection. Given the reach of the HSV-2 epidemic, and the contribution of HSV-2 to HIV infection, we must be creative in our approach to this most challenging chronic viral infection.

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