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Vaccines Against Genital Herpes

Progress and Limitations

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Abstract

Herpes simplex viruses (HSV) cause lifelong persistent infections with numerous disease manifestations. Genital herpes infections are widespread in populations throughout the world and a vaccine to protect against or subdue established genital herpes infections has been under development for decades.

Vaccine-mediated protection against persistent viral infections can be extremely difficult to achieve. The more rapidly a virus reaches its target tissue for persistence, the more vigorously a vaccine-induced immune response must defend the vaccinated individual. After exposure to HSV through sexual contact, only a few days are required for the virus to establish latent infection of its host. Despite numerous improvements, traditional vaccine approaches of whole virus or protein subunits have met with only marginal success.

The many disappointments have heightened interest in determining correlates of immune protection, studies pursued both in animal models and in humans. They have also led to reassessment of the goals of vaccination. Necessity has sparked several creative new vaccine approaches involving nucleic acid or live attenuated viruses and vectors. With improved concepts of protective immune responses has come fervent discussion of the means to stimulate and maintain cell-mediated immunity. The result of this work is likely to be a more thorough understanding of antiviral immunity in the genital mucosa and the nervous system, and of HSV pathogenesis and immune evasion strategies, as additional strides are taken toward the goal of a successful vaccine with which to confront HSV.

Herpes simplex virus 2 (HSV-2) is most often the cause of genital herpes, a sexually transmitted disease that occurs throughout the world. The first description of the disease dates to more than 2000 years ago, and the virus is likely to have co-evolved with the human population for many more millennia. This long period of adaptation to human predation makes HSV one of the more formidable viruses to counteract by prevention or therapy. It has developed an extensive array of offensive and defensive tactics to ensure successful infection of individuals and stable maintenance in the human population. These tactics, along with methods of broad and balanced immune response induction, must be considered in development of successful prophylactic and therapeutic vaccines.

1. Epidemiology and Disease

HSV-2 can be detected serologically in approximately 22% or 45 million persons in the US aged 12 years and older.[1,2] The virus is not constrained by gender, age or socioeconomic boundaries, al-

though risk of acquisition correlates most strongly with female gender and number of sexual partners.[2-5] Moreover its incidence, which rose dramatically in the 1970s and 1980s[6] continues to climb.[1,2] The prevalence of the virus is even greater in developing countries, as exemplified by Uganda where seropositivity is estimated at 72% among women.[7] The presence of HSV genital lesions is also a risk factor for acquisition or transmission of HIV through sexual contact.[8-10]

HSV probably gains entry to a new host through abrasions of the vaginal mucosa, anal mucosa or penile skin as a result of sexual activity. During primary genital infection, or initial genital infection subsequent to primary oral infection with HSV-1, scattered pustules, vesicles and ulcerative lesions may develop at the site of contact. Other common symptoms include pain and itching, urethral and vaginal discharge, dysuria and inguinal adenopathy. A variety of systemic symptoms such as photophobia, fever, headache and neuralgias may accompany acute infection and, rarely, acute HSV-2 encephalitis ensues. Within days of establishing infection in the genital skin or mucosa, the virus enters sensory nerve terminals innervating the mucosal epithelium and is transported retrogradely to the nerve cell body (figure 1). Here the virus establishes a latent infection, ensuring a permanent relationship with its host. During latency, the viral DNA genome is harboured in the sensory nerve and transcription from the viral genome is drastically reduced. Consequently little if any viral protein is synthesised to signal the virus' presence to the immune system,[11] and thus it remains safe from elimination. Periodic stressors such as menses, fever and corticosteroid use signal the virus to regain transcriptional activity and reactivate. Infectious virus is produced that returns to the mucosa via axonal transport, where recurrent lesions may develop (figure 1). Because a new round of latent infection is simultaneously established, HSV-2 persists for the lifetime of the infected individual.

HSV-2 causes the majority of genital herpes infections, but HSV-1 has been identified as the aetiologic agent in 10 to 40% of persons.[12] The symptoms of acute infections with HSV-1 and HSV-2 are similar, but infections with HSV-2 are usually more severe and reactivate with 16-fold greater frequency.[12] Remarkably, however, many

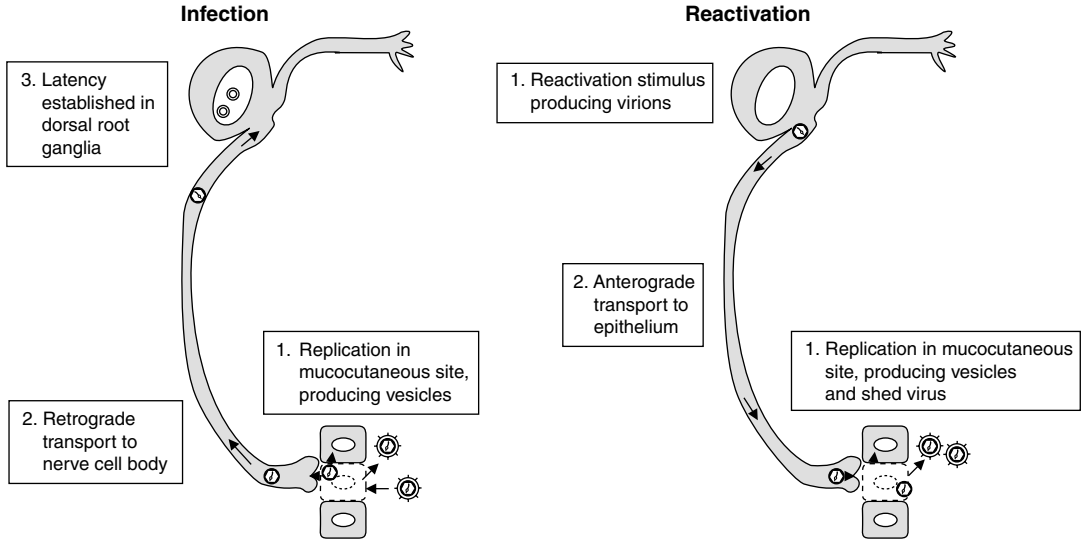

Fig. 1. Routes of herpes simplex virus infection and reactivation.

Table I. Historical vaccine formulations: results of human trials

Designation	Type	Possible reasons for failure
Lupidon H, G	Formalin- or heat-inactivated tissue homogenate	Safety concern; low immunogenicity; poor cell-mediated immune induction
Cappel, Dundarov	Detergent disrupted virion envelope proteins	Low immunogenicity; poor cell-mediated immune induction
Skinner, Kutinova	Formalin-inactivated, detergent extracted virion proteins	Some degree of efficacy in therapeutic trial
Merck GS	Virion-derived, purified glycoprotein + alum	Low immunogenicity; narrow spectrum immune response
Chiron	Recombinant gD/gB + MF59 adjuvant	Immunogenic but did not prevent seroconversion
Pasteur Merieux Connaught R7020	Live attenuated, replication-competent	Safety plus stability concern; insufficient immunogenicity in naïve vaccinees; strong inflammatory reaction in immune vaccinees

gD/gB = glycoprotein D/glycoprotein B.

if not most seropositive individuals experience subclinical infection. Asymptomatic shedding occurs in the majority of all genital infections[13-16] and because most disease transmission is associated with asymptomatic shedding[16,17] the virus moves surreptitiously through an unwary population.

One of the most compelling arguments for prophylactic vaccination to prevent HSV genital infection is the prospect of transmission from infected mothers to neonates. Neonatal infections usually occur by contact with virus in maternal secretions during birth, and risk for transmission is greatest if the mother acquires primary or initial infection near the time of labour.[18] These infections affect approximately 1 in 3500 newborns in the US,[19,20] with fewer in Western Europe[21,22] but significantly higher rates in developing countries. Infections of the neonate can be confined to skin, eyes and mouth, to the nervous system, or can be widely disseminated. The risk of neurological sequelae and death increases in proportion to degree of dissemination.[23-25]

2. Clinical Vaccine Trials

Vaccination to prevent HSV infections, or to reduce reactivation frequency and severity, would reduce morbidity, the risk of dissemination to newborns and the risk of HIV acquisition. An effective vaccine would also curb the continued increase in transmission to uninfected individuals, either by protecting them directly or by reducing virus shedding from infected partners. Vaccines, then, may be sought for two independent purposes: prophylaxis in uninfected individuals and therapy in those already infected.

Vaccine development against HSV has a history stretching back more than 70 years. Many types of vaccines have been evaluated in clinical trials of immunogenicity and prophylactic or therapeutic efficacy (table I). Some of the early vaccines appeared immunogenic but often the trial design precluded interpretation of efficacy. The Skinner vaccine has been shown in placebo-controlled clinical trials to have marginal therapeutic efficacy in reducing severity of recurrences[26] and further evaluation is under consideration.

Subunit vaccines prepared from recombinant glycoproteins became available with the advent of genetic engineering. These vaccines are safer and easier to produce. Some of the individual glycoprotein preparations, however, were poorly immunogenic and did not protect against transmission.[27] Of interest, those individuals vaccinated with glycoprotein subunit who subsequently acquired infection developed unusual symptoms, suggesting that the immune responses induced by the vaccine were skewed compared with naturally acquired immunity and that this skewing had pathological consequences.[28] Later formulations induced antibody responses to glycoprotein (g) D, gB or gC that met or exceeded serum antibody titres seen in infected individuals.[29,30] However, HSV attachment to cells is a complex process[31] making it unlikely that antibody response to a sin-

gle glycoprotein would prevent infection. In addition, cell-mediated immune responses have been demonstrated but reliance on a handful of epitopes from a single protein may be limiting. In Phase III clinical trials, these individual glycoprotein vaccines failed to protect individuals from infection and disease.[32] Thus, reasons for the failure of these products may be several, and their failures demonstrate the intricate relationship between the types of immune responses induced and the biology of the virus.

Live attenuated HSV vaccines were also developed by engineering deletions in known virulence genes, and inserting additional copies of genes encoding gB and gD.[33] The live attenuated virus approach would in theory circumvent the problem of narrow spectrum immune responses elicited by glycoprotein subunits and would induce cell-mediated immune responses. Clinical trials were halted, however, when it became apparent that these viruses provoked strong inflammatory reactions in previously immune individuals and inadequately stimulated immunity in naïve individuals.[34]

3. Reassessment

3.1 Correlates of Protection

The apparent failure of earlier vaccine approaches has renewed interest in determining correlates of immune protection to guide the informed design of future vaccine candidates. Both antibody and T cells could be envisioned to have roles in intervention, antibody by neutralising extracellular virus, fixing complement or arming killer cells, and T cells by eliminating virus-infected cells before progeny virus is produced and suppressing lytic replication in neurons. HSV-2 infections are often more severe in individuals with T cell suppression than in those with deficits in humoral immune responsiveness. These experiments of nature suggest that cell-mediated immune responses may be of primary importance in preventing severe primary or recurrent infection with HSV-2. While little is known regarding T cell activity in the nervous systems of HSV-infected individuals, HSV-specific T cells have been observed to persist in the genital tract of women infected with HSV-2.[35] Both CD4+ and CD8+ T cells are present in lesions[36-38] and clearance of virus correlates with infiltration of HSV-specific cytotoxic T lymphocytes (CTL)[37,39] and interferon (IFN)-γ producing lymphocytes.[40] HSV-specific CD4+ and CD8+ T cells isolated from HSV lesions have been shown to recognise capsid and tegument proteins as strongly as the glycoproteins[41-43] providing one possible basis for the insufficient cell-mediated immunity and efficacy induced by glycoprotein vaccination.

Infection of mice and guinea pigs with HSV-2 has been used extensively to model genital herpes infection and disease for the purposes of vaccine testing, and to examine the role of various immune components in resistance. In the mouse model, HSV immune cell-mediated responses provide significant protection against subsequent challenge of the genital mucosa with HSV.[44-47] Most investigators argue in favour of CD4+ T cell involvement in protection, although a principal role for CD8+ T cells has been espoused by others.[44] Both serum-derived and mucosally produced immunoglobulin (Ig)G have virus neutralising properties,[48] and play an important role in protection of the mucosa and nervous system,[49,50] particularly in conjunction with immune T cells.[47] Interestingly, pre-existing HSV-specific IgA does not contribute significantly to protection of the murine vaginal mucosa,[49,51] which may also be true in humans.[52]

These data, together with human studies, suggest that an optimal vaccine will induce both humoral and cellular immune responses, in proportions mimicking natural infection. Clearly, then, development and testing of new types of vaccine that stimulate cell-mediated immune responses is warranted, as is further investigation of immune correlates of protection at mucosal surfaces and within the nervous system.

The question then becomes how best to induce and maintain cell-mediated immune responses?

Current obstacles to vaccine-mediated elicitation of T cell responses are several. Firstly, very few epitopes have been identified in only a limited number of HLA types. Secondly, memory immune T cells exist in a quiescent state and, unlike circulating immune antibody, recall from memory to activated effectors upon antigen re-exposure requires 2 to 3 days.[53] Lastly, in the case of vaccines as therapeutic agents, it may be difficult to redirect an aberrant or insufficient immune response[53] even if the source of the aberration is identified. These are formidable challenges that we now know face development of the next generation of candidate vaccines.

It is interesting to note that the types of immune responses most valuable in neutralising virions upon exit from the nerve termini into intercellular space (antibody) are different than those required to eliminate initial mucosal lesions (CTL). Thus, an optimal therapeutic vaccine may differ in composition from an optimal prophylactic vaccine. Indeed, glycoprotein subunit vaccines, although showing only meagre success in reducing transmission to naïve individuals, generally have shown greater promise as therapeutic agents.[32,54,55] High antibody titres in women of childbearing age infected with HSV-2 also appear desirable to guard against transmission from mother to infant.[56]

3.2 Goals of Vaccination

Once within the neuron, HSV is virtually inaccessible to immune responses. An effective prophylactic vaccine, then, would be one able to interrupt virus infection prior to entry into the nervous system. Complete immunological prevention of nervous system infection, however, may be exceedingly difficult to achieve. A more realistic goal of reducing establishment of latent infection by eliminating as many virus-infected cells and cell-free virions as possible has been largely accepted. The apparent correlation between the severity of primary infection and the frequency and severity of recurrent infections[57] support this modified goal of prophylactic vaccination.

3.3 Safety Versus Efficacy

Unfortunately in the case of herpesvirus vaccines, safety and immunogenicity often appear reciprocally related, and thus a balance between the two must be struck. The central issue appears to be stimulation of cell-mediated immune responses. Glycoprotein vaccines, although they can be used safely, require adjuvant to stimulate T cell responsiveness. Live attenuated viruses, while stimulating broader cell-mediated immunity, suffer from concerns regarding reversion to virulence, residual pathogenicity and capacity to establish latent infections. Future vaccine designs must convey superb immune stimulating capacity while ensuring the safety of naïve, previously virus-exposed and mildly immunocompromised vaccinees.

4. Progress and Prospects

In general there are two types of vaccines currently under development, those composed of protein or nucleic acid and those composed of live but genetically altered virus (table II). Necessity has sparked some ingenuity within both of these categories.

4.1 Protein-Based Vaccines

For glycoprotein subunit vaccines, new adjuvant formulations are providing improved immunogenicity while minimising inflammatory reactions. One new vaccine combining gD in the adjuvant monophosphoryl Lipid A (MPL) was recently reported to have achieved efficacy in prophylaxis against disease in humans (although not infection and only in female vaccinees).[58]

Detergent solubilised HSV-2 antigens mixed with Quil A to form immunostimulating complexes (ISCOMS) have been shown in mice to stimulate strong humoral and cellular cytokine responses.[59] Guinea pigs prophylactically immunised with ISCOMs have reduced incidence and severity of genital lesions and incidence of spontaneous shedding after HSV-2 genital challenge.[60]

Table II. Advantages and limitations of current vaccine prototypes

Vaccine type	Potential advantages	Potential disadvantages
Glycoprotein + adjuvant	Safety	Multiple doses; limited immune responses
ISCOMS	Multiple proteins incorporated	Multiple doses
Plasmid-based	Cost-effective; versatile	Multiple doses; safety unproven; best with protein boost
Virus vectors	Endogenous synthesis of viral protein	Limited immune responses
Replication-compromised virus	Broad spectrum immune responses	Establishment of latency
Replication-defective virus	Broad spectrum immune responses; essentially no latency	Multiple doses?

ISCOMS = immunostimulating complexes.

In addition to prophylaxis, the safety and humoral immune-stimulating capacity of protein subunit vaccines may make them ideally suited for increasing antibody titres in pregnant women to maximise fetal protection.

4.2 DNA-Based Vaccines

The infinite malleability of DNA combined with greater understanding of the immune response has led to a versatile new approach employing immunisation with genetic material.[61,62] The plasmids employed encode immunogenic proteins or epitopes of HSV proteins *in vivo*. DNA-based vaccination could theoretically be used to stimulate the immune system with mixtures of multiple plasmids encoding a variety of peptides and proteins. Immunisation of mice and guinea pigs with plasmids encoding various individual glycoproteins has elicited CTL and antibody responses, and reduced acute disease upon challenge.[61,63,64] In practice, however, it appears that multiple immunisations with large quantities of nucleic acid are required to stimulate a response and the longevity of the response has not yet been ascertained. A recent comparison of recombinant gD protein with gD-encoding plasmid immunisation showed superior protection against high-dose challenge elicited by the protein.[65] Similarly, immunisation with vaccinia vectors encoding gC or gE protected mice more completely than gC or gE plasmid immunisation.[31]

DNA encoding peptide conjugates that incorporate both CD4+ and CD8+ T cell epitopes have also been developed. These heteroconjugates augment the efficiency of CTL induction, and thereby increase the protective capacity of CTL peptide vaccination. For this type of vaccine to be effective, broadly immunodominant T cell epitopes as well as human leucocyte antigen (HLA) supermotifs must be identified to permit design of peptides that can be presented to T cells by a variety of HLA alleles.[66,67] Recent work by Koelle and Kwok has allowed great progress toward identifying CD4+ T cell epitopes recognised in the context of HLA class II molecules.[68,69] Whether sufficient immunogenicity can be obtained using DNA encoding peptide conjugates remains to be determined. In general, it appears that DNA vaccines can be safely used in humans but safety is as yet unproven.

4.3 Live Virus Vectors

Live attenuated virus vectors, including vaccinia and adenovirus expressing HSV gB or gD[70,71] or infected cell protein (ICP)27[72] have been constructed. They stimulate protective immune responses in animals, but enthusiasm for them as HSV vaccines has been dampened by safety concerns. Secondly, individuals already immune to the vector by way of previous smallpox vaccination or natural infection may efficiently eliminate the vector before immune responses to the vector-encoded HSV protein can be mounted. Finally, these vaccine forms suffer some of the shortcomings of the glycoprotein subunit vaccines in that the HSV-specific immune responses provoked are limited in scope largely to a single protein or epitope. New attenuated virus vectors are

under development that may circumvent certain of these obstacles.

4.4 Live Replication-Deficient HSV

The use of live attenuated HSV for vaccination holds the advantages of stimulating cellular and humoral immune responses to a wide variety of viral proteins – reflecting a natural spectrum of responses – and amplification of the vaccine in the immunised individual. A new generation of replication-defective and single-cycle virus vaccine prototypes that promise increased safety has evolved from the live attenuated virus vaccine genre.

HSV-2 genetically mutated in the locus encoding gH undergoes a single round of productive infection in the immunised host. The virions produced, because they lack a protein essential for viral entry, cannot infect a second round of cells. This type of mutant virus stimulates serum antibody responses after parenteral or mucosal immunisation of guinea pigs,[73] reduces primary infection and ameliorates recurrent disease symptoms.[74] This so-called DISC virus is undergoing Phase I trials in seropositive volunteers. In a comparison of approaches, DISC viruses were shown to stimulate levels of antibody equivalent to ISCOMS and to protect mice equivalently from lethal intraperitoneal challenge.[75] A potential disadvantage of DISC vaccine, however, is the capacity of this mutant to establish latency in the nervous system. Although it cannot reactivate, the viral nucleic acid will be maintained long-term in neurons of the vaccinated host.

Alternative HSV-2 mutants have been developed that are replication-defective by virtue of deletion in the ICP8- and/or ICP27-encoding loci.[76,77] These viruses synthesise the majority of viral proteins, including gB and gD, in infected cells and possess an extra measure of safety because they establish latency with extremely low frequency.[47,77] The immune responses induced by replication-defective viruses are qualitatively and quantitatively different from those elicited by immunisation with UV-inactivated virus[40] indicating that proteins synthesised *de novo* in infected cells are largely responsible for immune induction. Their capacity to elicit adequate immune responses is a matter of concern, but it was recently shown that deletion of virion host shutoff (vhs) function in addition to ICP8 improved the immunogenicity of replication-defective HSV to the level of an attenuated, replication-competent virus.[78]

4.5 Combined Modalities

In many ways the challenges that face vaccine development against HSV equate with those facing vaccine design against HIV. One promising strategy employed recently in HIV vaccinology is that of immunising with one reagent and boosting with a heterologous preparation. This approach was recently adopted to demonstrate the enhancement of both antibody and T helper cell (Th) responses by HSV glycoprotein DNA prime, protein boost[79] or vaccinia vector boost[80] regimens.

5. Immune Modulation

5.1 Neutralising Virus Defences

Herpes simplex viruses, as all the herpes viruses, have acquired or evolved a number of strategies to circumvent both innate and acquired host defences. Perhaps most important among these from the standpoint of vaccination are the inhibitory effects of ICP47 on the major histocompatibility complex (MHC) class I pathway of antigen presentation,[81] the more global effect of vhs on immune induction,[82] and interference with dendritic cell activation and maturation.[83] While not absolute mechanisms of immune avoidance, these virus strategies may have an impact on the magnitude or type of immune responses that are evoked, particularly in cases where antigen may be limited. Further research into the importance of these mechanisms in immune response inhibition is warranted to ascertain their significance to the design of live virus vaccines.

5.2 Augmenting Immune Responsiveness

Research is uncovering not only mechanisms by which HSV-2 effects its replication and persistence programme, but also mechanisms by which an effective immune response to the virus is induced. Better understanding of the interplay of various chemokines and cytokines has revealed the beneficial influence of type 1 cytokine-driven responses in protection of the genital tract,[75] and has led to promising vaccine approaches in which individual cytokine and chemokine genes are encoded by the vaccine virus[84] or are included as plasmids along with DNA specifying immunogenic viral proteins.[85-88] Costimulation molecules are another component essential to induction and guidance of effective immune responses. Here, too, plasmids encoding B7 costimulation molecules have been shown to improve the magnitude of potential vaccines against HSV-2.[89] In like fashion, expression of certain accessory or adhesion molecules, such as lymphocyte function-associated antigen (LFA)-3,[90] provides an exciting opportunity to improve the induction of vaccine-specific immune responses. But whereas some cytokines and chemokines augment the potency of potential vaccines, others increase mortality upon challenge.[86] Thus, there are many important details left to learn about specific molecules and inflammatory reactions between viral antigens and the immune system.

5.3 Mucosal Vaccination

Information about the genital mucosa as an immune response organ lags behind our understanding of other mucosal surfaces. The observation that mucosal immunisation may be able to boost systemic responses and/or provide an additional line of defence has yet to be adequately explored and may be particularly important.[80,91,92] Certain adjuvants may improve mucosal immune responses. For example, oligodeoxynucleotides containing CpG motifs of *Escherichia coli* have been shown to dramatically stimulate IgA responses to gB in the genital tract after intranasal inoculation and to increase protection against lethal vaginal challenge with HSV-2.[93] Because HSV-2 rapidly gains access to its site of latent infection, it seems sensible that vaccination provide effectors that are present in the mucosa and can be rapidly recalled to combat infection when HSV-2 is encountered. On this topic and many others there is much left to learn.

6. Conclusion

Development of a prophylactic and therapeutic vaccine against HSV has expanded from inactivated virus and protein subunits to nucleic acid and engineered attenuated virus mutants and vectors. Progress toward a vaccine has been slow because of the many obstacles posed by the virus – multiple proteins involved in cell attachment and penetration, immune evasion tactics and rapid entry into the nervous system. Progress has also been limited by the very nature of cell-mediated responses that must be recalled from memory in distant locations. But greater understanding of what a vaccine must be capable of, and new insights into ways to induce and modulate these immune responses through novel vaccine formulations, are being brought to bear on the problem and suggest exciting prospects. Like many fields, that of herpes vaccine development is accelerating toward its goal of an effective vaccine with adequate safety for prophylactic and therapeutic use.

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