Antigenic breadth: a missing ingredient in HSV-2 subunit vaccines?


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The successful human papillomavirus and hepatitis B virus subunit vaccines contain single viral proteins that represent 22 and 12%, respectively, of the antigens encoded by these tiny viruses. The herpes simplex virus 2 (HSV-2) genome is >20 times larger. Thus, a single protein subunit represents 1% of HSV-2’s total antigenic breadth. Antigenic breadth may explain why HSV-2 glycoprotein subunit vaccines have failed in clinical trials, and why live HSV-2 vaccines that express 99% of HSV-2’s proteome may be more effective. I review the mounting evidence that live HSV-2 vaccines offer a greater opportunity to stop the spread of genital herpes, and I consider the unfounded ‘safety concerns’ that have kept live HSV-2 vaccines out of U.S. clinical trials for 25 years.

KEYWORDS: alum + monophosphoryl lipid A adjuvant • antigenic breadth • deletion of ICP0’s nuclear localization signal • glycoprotein D • herpes simplex virus 2 • infected cell protein 0 • live HSV-2 vaccine • plaque-forming unit • severe-combined immunodeficiency

Expert commentary

Prologue

Herpes simplex virus 2 (HSV-2) vaccine reviews often provide an overview of which vaccine approaches have been considered in recent years [12]. The current review focuses on what I believe is a more pressing question: Why do promising HSV-2 vaccines keep failing in clinical trials [3–9]? What doctors and the general public desire is a HSV-2 vaccine that works. What scientists desire is a better understanding of how to separate the wheat from the chaff when it comes to HSV-2 vaccines. The intention of this review is to consider such matters.

I hope to make plain that ‘antigenic breadth’ is a critical concept in HSV-2 vaccine efficacy, but has slipped under scientists’ radars for too long. Although vaccine scientists have been testing HSV-2 vaccines for three decades [10,11], the spread of HSV-2 genital herpes has not been slowed. Millions of our children will suffer the same fate unless we advance an effective HSV-2 vaccine into clinical trials posthaste.

The key questions are, ‘Is HSV-2 genital herpes a vaccine-preventable disease?’, and if so, then ‘Which HSV-2 vaccine approaches are most likely to achieve this goal?’ Against this backdrop, I discuss what I believe has gone awry with past HSV-2 vaccine strategies and consider what we might do differently in the future to improve our odds of success.

Introduction

The field of herpes immunology informs us how the innate and adaptive immune responses work together to stop the spread of herpes simplex virus in vivo [12]. As someone who has studied herpes immunology for 20 years, I find the rationale behind most ‘promising’ HSV-2 subunit vaccine concepts [3,13] to be inconsistent with my understanding of how animals acquire immunity to HSV-2 and drive the virus into a state of latency.

The advantage of protein subunit vaccines is their extraordinary safety; they cannot produce the symptoms of herpetic disease. Dozens of permutations of the HSV-2 subunit vaccine approach have been suggested. In particular, HSV-2 glycoproteins B or D (gB or gD) have been extensively considered as subunit vaccine antigens. Studies of gD subunit vaccines in animal models in the 1980s [14], 1990s [15], 2000s [16,17] and 2010s [18,19] suggested that gD subunit vaccines should be sufficient to prevent HSV-2 genital herpes. Unfortunately, this prediction...
has not been borne out by the data from six human clinical trials [4–9].

In particular, I question the premise that HSV-2 vaccines containing 1% of HSV-2’s proteins (Figure 1A) should elicit 100% of the protection against genital herpes that is attainable. Immune responses to HSV-2 involve B- and T-cell responses to >20 viral proteins [20,21]. In particular, investigators at the University of Washington have assembled an impressively detailed description of the polyclonal CD4+ and CD8+ T-lymphocyte response that humans mount to HSV-2 infection, which may be directed against up to 30 HSV-2 proteins [22–25]. Likewise, earlier studies by this same group suggested that the human antibody response to HSV-2 was directed against more than a dozen HSV-2 proteins [26–28]. In contrast to the natural, highly polyclonal B- and T-cell responses to HSV-2, most HSV-2 vaccine candidates under study contain just one or two viral proteins [4–9] or a collection of HSV-2 peptides [29].

In this review, I advance a novel hypothesis that has not been carefully evaluated in the field of HSV-2 vaccine research. Specifically, I propose that ‘antigenic breadth’ is a simple and unifying concept that may explain why past HSV-2 subunit vaccines have failed and why a live HSV-2 vaccine would be more likely to prevent HSV-2 genital herpes in the human population. I define the term antigenic breadth as the percentage (%) of an infectious agent’s proteome that is present, or may be expressed, by a given vaccine candidate, and which may thus serve as the basis for immunogenic stimulation and clonal expansion of B- and T-cells (i.e., the foundation of vaccine-induced protection).

It seems logical that a vaccine lacking 99% of HSV-2’s antigens may have limitations. For example, if the bone marrow produces naive, mature B- and T-cells with the potential to respond to 30 of HSV-2’s proteins, then a subunit vaccine strategy based on a single HSV-2 protein may only drive clonal expansion of approximately 3% of the adaptive immune defenses available to control HSV-2. Consistent with this hypothesis, HSV-2 subunit vaccines have been failing in US clinical trials for 25 years [4–9,13,28]. Genocea’s GEN-003 vaccine [30] and Agenus’s HerpV vaccine [29] are the latest iteration of the concept [31,32]. It has been suggested that these latest HSV-2 subunit vaccines will be different. However, similar suggestions have been made repeatedly for 30 years [33–42], and yet we still lack an effective HSV-2 vaccine.

Live HSV-2 vaccines are a logical alternative and offer a simple and feasible means to expose a vaccine recipient’s B- and T-lymphocytes to 99% of HSV-2’s peptide antigens (Figure 1B). In animal studies, live HSV-2 vaccines elicit robust protection against HSV-2 genital herpes in the hands of several investigators [43–48]. However, enthusiasm for testing and developing of live-attenuated HSV-2 vaccines has been curbed by suggestions that a live, replicating α-herpesvirus vaccine may be intrinsically unsafe. Such safety concerns underlie the rationale to develop ‘safer’ approaches, such as Sanofi Pasteur’s replication-disabled HSV-2 ACAM-529 vaccine candidate [49] and other replication-disabled HSV-2 vaccine candidates [50–52]. As I discuss in depth in Section ‘Live HSV-2 vaccines: an unexplored opportunity to prevent genital herpes’, although safety concerns have been repeatedly raised about live-attenuated HSV-2 vaccines, there is a dearth of evidence to support these claims.

With regard to the potential safety of a live HSV-2 vaccine, it is relevant to note that HSV-2 shares approximately 60 homologous genes and a neurotropic-life cycle in common with varicella-zoster virus (VZV) [53]. The live-attenuated VZV Oka vaccine has been safely used in >50 million recipients [54,55] and has been effective in preventing chickenpox and shingles. If a live VZV vaccine is feasible, then it is unclear why a live-attenuated variant of the homologous HSV-2 virus should not...
be equally feasible. Moreover, >3 billion people are currently infected with wild-type HSV-1 or HSV-2. It is difficult to imagine a scenario in which a live-attenuated HSV-2 vaccine would pose a greater health risk to the human population than the continued, unchecked spread of wild-type HSV-2.

A major objective of this review is to compare subunit vaccines versus live HSV-2 vaccines and analyze how they square with herpes immunology. Based on this analysis, I offer two major conclusions. First, the failure of HSV-2 subunit vaccines in clinical trials should no longer be deemed ‘surprising’; subunit vaccines have been failing for 25 years. Second, we have learned in recent years that live HSV-2 vaccines are 50 times more effective than subunit vaccines in side-by-side tests; perhaps it is time to evaluate their potential to stop the spread of genital herpes in one or more human clinical trials.

**Recurrent HSV-2 genital herpes & the need for a vaccine**

The disease of genital herpes may be caused by HSV-1 or HSV-2. Although ‘only’ 10–20% of HSV infections produce symptomatic disease, the number of persons who live with the symptoms of recurrent herpetic disease represents a population greater than the United States. Thus, herpetic disease remains a major, unresolved health issue.

Seroepidemiology indicates that 8–16% of the human population is infected with HSV-2. Between 0.5 and 1.1 billion people harbor persistent HSV-2 infections and serve as a vast reservoir for the transmission of HSV-2 to future sexual partners. A total of 10–20 million naive individuals acquire new HSV-2 infections each year. The frequency of HSV-1 infection is higher; somewhere between 30 and 70% of the human population is infected with HSV-1, which means that 2–5 billion people carry HSV-1.

HSV-2 is better adapted to complete its life cycle in the human genitalia. HSV-2 efficiently reactivates in sacral ganglia that innervate the genital region, and thus latent HSV-2 infections commonly cause recurrent genital herpes. Although HSV-1 accounts for one-third of cases of primary genital herpes, latent HSV-1 infection of the sacral ganglia is less likely to successfully reactivate and cause recurrent genital herpes. Thus, most vaccine efforts have focused on HSV-2 because it causes a larger burden of recurrent genital herpes.

HSV-2 infection produces a spectrum of clinical outcomes. If we assume 1 billion people are infected with HSV-2, then approximately 800 million harbor asymptomatic infections, whereas approximately 40 million individuals live with genital herpes that recurs more than once per year. HSV-2 genital herpes has been aptly described as a ‘silent epidemic’; herpes sufferers typically conceal the condition from their friends and family. Life-threatening consequences of HSV-2 infection are less common but include neonatal herpes resulting from transmission to newborns during vaginal delivery. Moreover, HSV-2-infected individuals are threefold more vulnerable to infection with human immunodeficiency virus. For these reasons, it is widely agreed that an effective HSV-2 vaccine is an important and medically relevant goal.

**What is the goal of a HSV-2 vaccine?**

When asking the question, ‘Is a HSV-2 genital herpes vaccine feasible?’ it is relevant to first define the desired endpoint of an effective HSV-2 vaccine so that the criteria for success may be clearly understood by all stakeholders including patients and doctors. In my opinion, the goal of a HSV-2 genital herpes vaccine should be to prevent vaccine recipients from developing the symptoms of genital herpes disease following an exposure to HSV-2.

It has been suggested the goals of a HSV-2 vaccine should include rendering vaccine recipients impervious to infection with HSV-2. For example, the review of Dropulic and Cohen (2012) includes the statement, ‘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’. Likewise, the review of Johnston et al. makes a similar point.

In my opinion, sterilizing immunity against HSV-2, or any infectious agent, is simply not a realistic goal, and discussions of the topic represent little more than flights of fancy. The term ‘sterilizing immunity’ implies that a vaccine recipient’s adaptive immune defenses against a microbe may be heightened to such a level that the human body may be rendered impervious to a microbe’s entry. Such claims contradict a century’s worth of knowledge that informs us how the adaptive immune system functions. Clonal expansion of microbe-specific lymphocytes renders our bodies resistant to infectious diseases by virtue of accelerating the rate of immune clearance, or control, of a specific pathogen. The vertebrate immune system may only respond to a microbial infection that is in progress. There is no basis in immunological theory, or evidence, to support the misconception that a vaccine regimen may render the immune defenses able to form an absolute shield against infection with HSV-2, or any other infectious agent. Like all vaccines, an effective HSV-2 vaccine may only accelerate the rate with which our adaptive immune defenses control the spread of a HSV-2 pathogen upon infection.

The available evidence suggests that a more realistic goal for an effective HSV-2 vaccine would be to reduce acute HSV-2 replication and shedding from the genital epithelium by 100- to 1000-fold following an exposure relative to what may transpire in an unimmunized individual (see Section ‘Is a live HSV-2 vaccine more effective than a gD subunit vaccine?’). Although a 100- to 1000-fold reduction in the magnitude of primary HSV-2 infection does not constitute sterilizing immunity, it should be sufficient to yield an asymptomatic, primary HSV-2 infection; little to no latent HSV-2 DNA in the dorsal root ganglia that innervate the genitalia; and little to no chance of asymptomatic HSV-2 reactivation or recurrent herpetic disease later in life.

**Is a HSV-2 genital herpes vaccine feasible?**

Several editorials on HSV-2 vaccines from the past decade beg the question, ‘Is a HSV-2 vaccine feasible?’ I offer
three lines of evidence that HSV-2 genital herpes is almost certainly a vaccine-preventable disease.

First, during my graduate studies, there were numerous occasions on which I attempted to re-inoculate HSV-1 infected mice with the same virus. All of my attempts to do so failed and served as my introduction to the phenomenon that HSV-1 latently infected animals were highly resistant to a second exposure to exogenous HSV-1 (unpublished data). I was unaware at the time, but I was re-treading the same ground covered by earlier investigators who had documented that HSV-1 latently mice and rabbits acquired a remarkable resistance to later challenge with HSV-1 or HSV-2 [75-78].

Second, two groups of investigators in the 1980s noted that mice inoculated with a live-attenuated HSV-1 ICP34.5 mutant virus [79] or HSV-2 thymidine-kinase mutant virus [80] acquired robust resistance to challenge with wild-type HSM-1 and HSV-2, respectively. Independent investigators later used mice immunized with the HSV-2 thymidine-kinase mutant virus as a model system to study the mechanisms of robust, vaccine-induced protection against HSV-2 vaginal challenge [47,80,81].

Third, multiple groups have recently reported robust, vaccine-induced protection against HSV-2 genital herpes in animals immunized with inactivated [82] or live HSV-2 viruses [43-45]. If an effective HSV-2 vaccine is so readily demonstrable in animal models, then it begs the question, ‘Why do human clinical trials of HSV-2 vaccines keep failing?’ Although live HSV-2 vaccines confer remarkable protection against genital herpes in animal models, inactivated or live HSV-2 vaccines have not been systematically evaluated in US clinical trials.

**HSV-2 vaccine strategies: in theory & in practice**

HSV-2 vaccines in theory

Three general strategies may be pursued to obtain a HSV-2 vaccine, and these are subunit vaccines based upon HSV-2 proteins or epitopes [17,18,83-87]; killed, or inactivated, HSV-2 virions [82,88,89]; or live, genetically engineered HSV-2 viruses bearing attenuating deletions [43,44,46,56,90].

**HSV-2 subunit vaccines**

Dozens of permutations of the HSV-2 subunit vaccine approach have been suggested. In some cases, HSV-2 antigens are whittled down to their immunologically active epitopes [29,87,91]; one such T-cell epitope vaccine recently completed testing in a US clinical trial [31]. HSV-2 gB or gD have been considered extensively as antigens, and a myriad of gB or gD delivery systems have been considered. For example, poxvirus vectors [92], adenovirus vectors [93,94], replication-defective HSV-1 vectors [85,95-97], *Listeria monocytogenes* [91], plasmid DNAs [98,100], lentiviral vectors [101], vesicular stomatitis virus [102], poliovirus [103], VZV [104], *Salmonella typhimurium* [105] and adjuvanted proteins [99,106,107] have all been proposed as a potential means to deliver HSV-2 gB or gD to vaccine recipients for the purpose of eliciting protective immunity against HSV-2. The best studied gD subunit vaccine is the N-terminal 302 amino acids of HSV-2’s mature gD protein adjuvanted with alum and monophosphoryl lipid A (MPL) [16,17,106,107], which Glaxo Smith Kline advanced to a large Phase III clinical trial under the brand name Herpevac [4,108].

The advantage of gB and/or gD vaccines is their extraordinary safety; they cannot produce the symptoms of herpetic disease. The disadvantage of subunit vaccines relates to the fact that HSV-2 encodes another 73 proteins, and at least one-third of these proteins are immunogenic [20-28]. If most of HSV-2’s antigens are absent from gB and/or gD subunit vaccines, then it logically follows gB and/or gD subunit vaccines may not elicit all of the protective immunity against HSV-2 that is possible.

**Whole vaccines: inactivated versus live HSV-2 vaccines**

Two HSV-2 vaccine approaches that better address the issue of antigenic breadth are inactivated HSV-2 virions and live HSV-2 viruses (Figure 1B). I do not consider inactivated HSV-2 virions in depth in this review, except to note that therapeutic immunization of genital herpes patients with an inactivated (i.e., killed) HSV-2 vaccine was found to yield reductions in genital herpes symptoms in several European trials [109,110]. While these results were not reproduced in a small US clinical trial [88], it is relevant to note that killed HSV-2 vaccines that employ newer adjuvants may be far more effective [82,111].

Unlike killed HSV-2 vaccines, live HSV-2 vaccines synthesize viral proteins inside the cells of vaccine recipients. This is relevant because *de novo* protein synthesis is necessary to efficiently deliver viral peptides into the MHC class I antigen presentation pathway [112,113]. Hence, live HSV-2 vaccines offer a greater opportunity to drive the clonal expansion of CD8+ T-cells than killed HSV-2 vaccines. Given that CD8+ T-cells are a critical effector of protective immunity against HSV-2 [114-116], within this review I focus on live HSV-2 vaccines as the primary alternative to the HSV-2 subunit vaccine strategy.

**Live HSV-2 vaccines**

Some live HSV-2 vaccine candidates retain their capacity to replicate in the cells of vaccine recipients, and I refer to these as ‘live-attenuated’ HSV-2 vaccines. The oral polio (Sabin) vaccine [117], mumps/measles/rubella vaccine [118] and VZV Oka vaccine [54] all replicate within cells of human vaccine recipients. In contrast, live ‘replication-disabled’ HSV-2 vaccines infect cells in vaccine recipients, but are incapable of completing a single cycle of viral replication [43,50,119-121]. Sanofi Pasteur’s ACAM-529 vaccine represents the best-known example of a live, replication-disabled HSV-2 vaccine candidate [43,119]. I use the term ‘live HSV-2 vaccine’ to encompass both approaches, and use the terms ‘live-attenuated’ versus ‘replication-disabled’ where it is relevant to distinguish between these approaches.

The theoretical advantage of a live HSV-2 vaccine relates to antigenic breadth. Unlike HSV-2 subunit vaccines, a live HSV-2 vaccine may express up to 99% of HSV-2’s proteins, and thus may drive clonal expansion of a broad repertoire of virus-specific B- and T-lymphocytes (Figure 1B). The theoretical
disadvantage of live HSV-2 vaccines is that, unlike subunit vaccines, a small risk remains associated with any immunization that contains millions of copies of an infectious virus. Granted, the human health risk associated with the injection of a replication-disabled HSV-2 vaccine like ACAM-529 [43,119] would be negligible, but it is impossible to categorically state that the approach is devoid of risk.

HSV-2 vaccines in practice
A PubMed literature search was performed in November 2013 as a means to gauge the relative level of effort invested into exploring killed HSV-2 vaccines, live HSV-2 vaccines or HSV-2 subunit vaccines over the past 25 years. A search conducted using the terms ‘herpes simplex vaccine’ retrieved 468 articles published between 1989 and 2013 that were relevant to HSV-2 vaccine development efforts. Of these 468 publications, 175 were deemed tangentially relevant because the topic of the paper was either HSV-1 vaccine-specific (123 papers; e.g., [57,124,125]), or HSV-2-vaccine-specific (123 papers; e.g., [122,123]). In both cases, the findings of these studies were at least 3 years removed from a viable HSV-2-specific vaccine candidate.

Of the remaining 293 articles on HSV-2 vaccines, nearly 100 were reviews or commentaries. Thus, the ratio of primary research articles to reviews is 2-to-1 in the HSV-2 vaccine field. Of 195 primary research articles published on HSV-2 vaccines between 1989 and 2013, 123 articles (63%) focused on gB and/or gD-based subunit vaccines (Figure 2). Another 27 publications focused on subunit vaccine approaches that included at least one HSV-2 protein other than gB or gD (Figure 2). Only 4% (8 of 195) of primary research articles published between 1989 and 2013 focused on killed HSV-2 vaccines, and 16% focused on live HSV-2 vaccines (Figure 2). Specifically, 21 publications (11%) focused on replication-disabled HSV-2 vaccines, and 9 publications (5%) focused on live-attenuated HSV-2 vaccines (Figure 2). The remaining 4% of publications focused on nonspecific treatments to reduce genital herpes symptoms, such as vaccinia virus [126], bovine mammillitis virus [127], yellow fever vaccine [128], live poliovirus vaccine [129,130] or immunostimulatory compounds (Figure 2) [131,132]. Thus, two-thirds of HSV-2 vaccine research publications over the past 25 years focused on gB and/or gD-based subunit vaccines. This trend continued in 2013 [30,100,133–137].

One caveat of this analysis should be noted; it is unlikely that the term ‘herpes simplex virus vaccine’ would identify every study published in the past 25 years that is relevant to HSV-2 vaccine research. For example, some authors may be reluctant to call something a ‘vaccine’ until it has been approved by the FDA. Thus, Figure 2 provides only an approximate metric of the level of effort invested in research and development of killed, live or subunit HSV-2 vaccines over the past 25 years.

Which HSV-2 vaccine strategies have advanced to US clinical trials?
Between 1989 and 2013, a subset of 11 research articles reported the results of HSV-2 vaccine testing in US clinical trials. An analysis was performed to compare the number of patients enrolled in these trials as a means to gauge the level of interest in advancing killed, live or subunit HSV-2 vaccines into clinical practice (Figure 3).

Recombinant gB and/or gD combined with adjuvants were the focus of six efficacy trials spanning 1990–2012 [4–9], and nearly 14,000 people were enrolled in these trials (Figure 3). Neither gB + gD vaccines, nor gD alone, offered a clear therapeutic benefit against HSV-2; hence, Glaxo Smith Kline discontinued efforts to develop the Herpevac vaccine in 2010 [108].

For every 37 persons enrolled in a US clinical trial of a gB- and/or gD vaccine, 1 person has been enrolled in a clinical trial of every other conceivable HSV-2 vaccine approach (Figure 3). Specifically, 313 persons were enrolled in a trial of a killed HSV-2 vaccine [88] and 74 persons were enrolled in a trial of a replication-disabled HSV-2 vaccine (Figure 3) [138].

No individual has been enrolled in a US clinical trial of a live-attenuated HSV-2 vaccine. However, herpes sufferers have been injected with other live, replicating microbes in attempts to reduce herpes symptoms; namely, the live Mycobacterium bovis/BCG vaccine [139–142], the live poliovirus vaccine [129,143–145], the live smallpox vaccine [146,147] and the live VZV vaccine [148]. In a US clinical trial in 1985, 155 individuals with HSV-2 genital herpes were injected with the BCG vaccine; injections with the antigenically-unrelated Mycobacterium bovis did not reduce the frequency or severity of these patients’ herpes outbreaks [139]. The BCG and smallpox vaccines are based on nearly wild-type microbes isolated from cows [149–151]. Although live BCG and smallpox vaccines were deemed ‘safe enough’ to inject into herpes sufferers [139,146], several live HSV-2 vaccine strains that are far more attenuated [46,56,57] remain to be tested in a human clinical trial.
The studies of Meigner and Roizman on the live R7020 virus [95,152] are sometimes cited as evidence that we have previously investigated live HSV-2 vaccines [273]. However, I note the conventional meaning of the term ‘live-attenuated virus’ typically refers to a virus derived from a wild-type isolate whose disease-causing potential has been attenuated due to one or more genetic mutations, but which retains its capacity to express most of the wild-type virus’s antigens. In contrast, R7020 was derived from HSV-1 strain F and was genetically modified to ectopically express HSV-2 glycoproteins G, J, D and I from the long-repeated region of the HSV-1 genome [95]. To date, viral vectors that ectopically express antigens from other infectious agents have failed in several clinical trials (e.g., Merck’s HIV gag, pol and nef-expressing adenovirus vectors [153-156]). Such viral vectors are not the same as bona fide live-attenuated viruses, which have yielded numerous clinically effective vaccines including those used to prevent yellow fever, poliomyelitis, mumps, measles, rubella, chickenpox, shingles and rotavirus-induced diarrhea. To date, no evidence has been offered that a HSV-2 glycoprotein-delivery vehicle, such as HSV-1 R7020, may elicit the same level of protection against genital herpes that follows from immunization with a bona fide live HSV-2 vaccine.

If we expand the geographic distribution of HSV-2 vaccine trials to include the entire world, then we must also consider the trials of Casanova et al. conducted in Mexico City [157] and the unpublished results of the Immunovex HSV-2 trial performed in the United Kingdom [158]. Only the results of Casanova were made public. Based on the n = 32 patients in this study [157], the central point of Figure 3 remains the same if we expand the scope of clinical trials to include the entire world; namely, 97% of participants in all HSV-2 vaccine trials conducted to date have been immunized with adjuvanted gD and/or gB, or its placebo control.

Antigenic breadth: a missing ingredient in HSV-2 subunit vaccines?

Considerable effort has been invested into improving gD vaccines by modifying adjuvants [17,19,159,160] or delivery systems [19,30,83,591-99,101-106]. In contrast, little effort has been invested in addressing the more basic question, ‘Should a vaccine based on 1% of HSV-2’s antigens elicit 100% of the protection against genital herpes that is possible (Figure 1A)?’

Hepatitis B surface antigen is the immunogen in the hepatitis B virus (HBV) subunit vaccine and represents 12% of the antigenic breadth of HBV [161]. Likewise, L1 is the capsid protein that self-assembles into virus-like particles in the HPV-16 vaccine; the L1 protein represents 22% of the antigenic breadth of HPV-16 [162]. Therefore, a single protein represents a significant fraction of the antigenic breadth of these tiny viruses; the HBV and HPV-16 genomes are 3.2 and 7.9 kilobase pairs in size, respectively.

HSV-2’s genome, which is 50 times larger than HBV, is a 154 kilobase pair DNA molecule, which encodes 39,107 amino acids of peptides distributed across 75 proteins. The Herpevac vaccine advanced by Glaxo Smith Kline [134] was based upon 302 amino acids derived from gD [107] and represents 0.8% of HSV-2’s antigenic breadth (Figure 1A).

Given the complexity of the combined B- and T-cell response that naturally drives HSV-2 infection into a state of latency, I wrote an editorial in 2006 in which I made the argument that gD-based vaccines might lack the antigenic breadth necessary to serve as effective HSV-2 vaccines [163]. The argument fell flat for lack of evidence to prove or refute the hypothesis. Fast forward 7 years; extensive side-by-side testing of live HSV-2 vaccines versus gD vaccines has been completed [43,45,46,59]. As predicted, a live HSV-2 ICP0 vaccine that encodes 99.3% of HSV-2’s proteins elicits greater protection. Below, I review the evidence that animals immunized with live HSV-2 vaccines are better protected against HSV-2 genital herpes than animals immunized with gD subunit vaccines.

Before proceeding, it is relevant to note that in some biological contexts, subunit vaccines based on a fraction of a microbe’s antigens may be highly effective. For example, less than 0.1% of the antigenic breadth of Corynebacterium diphtheriae, Clostridium tetani, Haemophilus influenzae type b or Streptococcus pneumoniae are present in the subunit vaccines that prevent diphtheria, tetanus and two bacterial pneumonias, respectively. However, the success of these subunit vaccines is context-dependent and is not applicable to a complex intracellular pathogen such as HSV-2. For example, the antibody response against diphtheria and tetanus toxins (elicited by the DTap vaccine) only has to neutralize these extracellular bacterial toxins to prevent the diseases of diphtheria and tetanus [164]. Likewise, an antibody response against polysaccharide-protein conjugate vaccines is sufficient to promote the opsonization and clearance of the extracellular bacteria Haemophilus influenzae type b and Streptococcus pneumoniae before they may cause pneumonia [165].
In contrast, HSV-2 is a complex intracellular parasite that hides most of its antigens from extracellular antibodies. Because the bulk of HSV-2′s genetic information and foreign antigens are hidden inside host cells, protective immunity against HSV-2 requires the involvement of T-cells [22,33,114,116,167]. In particular, CD8+ T-cells are a critical effector in the rapid detection of virus-infected cells (via peptide-loaded MHC class I), which is critical to host control of HSV-2 replication via the secretion of suppressive cytokines, such as IFN-γ, and cytosis of virus-infected cells [114,116,168-172].

**Is a live HSV-2 vaccine more effective than a gD subunit vaccine?**

Mouse studies of live HSV-2 0ΔNLS vaccine

A panel of HSV-2 ICP0 mutant viruses was constructed for the purpose of evaluating their safety [56] and efficacy [45] as live HSV-2 vaccines. The lead candidate to emerge from this analysis, HSV-2 0ΔNLS, was a HSV-2 ICP0 mutant virus that lacked 292 amino acids of ICP0 and retained the capacity to encode 99.3% of HSV-2′s antigens (FIGURE 1B). Testing demonstrated that HSV-2 0ΔNLS was replication-competent, interferon (IFN)-sensitive, avirulent in immunocompetent mice and achieved an optimal balance between attenuation versus capacity to stimulate a protective immune response [56].

Studies were conducted to determine whether mice immunized with the live-attenuated HSV-2 0ΔNLS vaccine were better protected against HSV-2 genital herpes than mice immunized with a Herpevac-like gD vaccine [45]. To this end, mice were immunized on days 0 and 30 in the rear footpads with culture medium, live HSV-2 0ΔNLS or gD + alum/MPL (FIGURE 4A). Antibody titers against gD were an average of 200,000 in gD-immunized mice, which is similar to other published studies [16,17,19]. On days 80, 90 or 100, mice were vaginally challenged with 500,000 pfu of wild-type HSV-2 MS, and the summed results are presented as follows.

Mice immunized with culture medium were considered to be immunologically ‘naive’ as they had not previously been exposed to any of HSV-2′s protein antigens. These immunologically naive mice shed high titers of HSV-2 MS from their vaginas postchallenge, whereas mice immunized with gD + alum/MPL shed threefold less HSV-2 per vagina than naive mice, and only 3 of 15 survived HSV-2 vaginal challenge (FIGURE 4B). In contrast, HSV-2 0ΔNLS-immunized mice shed 160-fold less HSV-2 per vagina than naive mice, and this additional 50-fold protection against HSV-2 vaginal replication was functionally relevant; 35 of 35 0ΔNLS-immunized mice survived vaginal challenge with no signs of disease (FIGURE 4B).

To visualize vaccine-induced protection against HSV-2, replicate mice from the same immunization groups were vaginally challenged with a luciferase-expressing HSV-2 challenge virus (FIGURE 4C). Naive mice failed to control the spread of luciferase-expressing HSV-2 between days 2 and 6 postchallenge and succumbed to disease (FIGURE 4C). Mice immunized with gD + alum/MPL exhibited significant reductions in luciferase expression (FIGURE 4C), but this was insufficient to spare them from disease and death. In contrast, HSV-2 0ΔNLS-immunized mice were completely resistant to luciferase-expressing HSV-2 challenge virus, and 100% of animals exhibited disease-free survival (FIGURE 4C).

Guinea pig studies of the live HSV-2 0ΔNLS vaccine

Similar side-by-side tests were performed in guinea pigs, and equivalent results were obtained (FIGURE 5) [58]. Specifically, naive guinea pigs shed high titers of HSV-2 MS from their vaginas at times postchallenge (FIGURE 5A), and all developed florid perivaginal disease that required their sacrifice (FIGURE 5B). Guinea pigs immunized with a gD + alum/MPL vaccine shed an average fourfold less HSV-2 per vagina than naive guinea pigs (FIGURE 5A), but 3 of 4 still developed perivaginal disease that required their sacrifice (FIGURE 5B). In contrast, guinea pigs immunized with a live HSV-2 0ΔNLS vaccine shed an average 200-fold less HSV-2 per vagina than naive guinea pigs (FIGURE 5A). This 50-fold increase in protection against HSV-2 vaginal shedding was functionally relevant as all 0ΔNLS-immunized guinea pigs were completely protected from perivaginal disease and death (FIGURE 5B).

Side-by-side tests by other research groups

Three other research groups have reported similar results in side-by-side tests of whole viral vaccines versus a gD + alum/MPL vaccine. Hoshino et al. were the first to demonstrate that a live, replication-disabled vaccine, dls5-29 (i.e., the precursor of ACAM-529) induced a threefold increase in HSV-2 neutralizing antibody relative to a gD-2 subunit vaccine [59]. Second, Morello et al. reported that inactivated HSV-2 virions + alum/MPL adjuvant provided robust protection in mice against HSV-2 vaginal challenge, whereas mice immunized with gD + alum/MPL were not well protected [82]. Finally, Sanofi Pasteur demonstrated that the live, replication-disabled HSV-2 ACAM-529 vaccine elicited approximately 100-fold greater protection against HSV-2 vaginal challenge in guinea pigs relative to a gD + alum/MPL vaccine [43].

In summary, mice and guinea pigs immunized with gD + alum/MPL adjuvant possessed protection against HSV-2 challenge that was statistically significant, but inadequate to spare vaccine recipients from disease and/or death. These results clarify that Herpevac-like gD vaccines are not only ineffective in human clinical trials [4-9], but are also ineffective in mice and guinea pigs when compared with killed or live HSV-2 viral vaccines [43,45,82].

**Why so different?**

Preliminary conclusions

Adjuvanted gD vaccines elicit a high titer of antibody against gD, which is 1 of the 10 glycoproteins embedded in the envelope of HSV-2 virions. It is not a coincidence that gD and gB are the attachment and fusion moieties that mediate virion entry into human cells [173], and also the major targets of vaccine development efforts (FIGURES 2 & 3). It is often suggested that vaccines that raise neutralizing antibodies against virion entry
glycoproteins may be sufficient to protect vaccine recipients against viral diseases. This is the underlying rationale behind viral vaccines that contain HSV-2’s gB and gD antigens, as well as vaccines that contain human immunodeficiency virus’s gp120 and gp41 antigens [174,175] or influenza virus’s hemagglutinin antigen [176].

In the context of HSV-1, neutralizing antibodies alone are insufficient to mediate a protective immune response, as was first shown in the 1970s [177,178]. The word herpes is derived from the Greek root herpein, which means ‘to creep’. Indeed, HSV-1 and HSV-2 are highly cell-associated viruses that creep from cell to cell. In theory, only a few milliseconds is required for an approximately 200 nm HSV-2 virion to transit across a narrow intracellular junction or neuronal synapse. This offers little opportunity for antibodies to neutralize HSV-2 virions as they exit one cell and transit several nanometers to the next cell in vivo. In contrast, poliovirus and HBV must spread via the bloodstream to cause disease; hence, vaccine-induced antipolio or antiehepatitis B antibodies offer a formidable barrier to the spread of these viruses.

If HSV-2 virion neutralization is insufficient to provide protective immunity against HSV-2, then it is unclear why serum levels of gD-specific antibodies should provide a reliable index of vaccine-induced protection against HSV-2 [58].

Immunofluorescent staining of HSV-2 plaques was initially used to explore this hypothesis. As expected, serum from naive
mice lacked HSV-2-specific IgG antibodies (left panel, Figure 6A). Serum from mice immunized with gD + alum/MPL possessed only low levels of antibody that bound the 75 viral proteins present in HSV-2− cells (middle panel, Figure 6A). In contrast, serum from HSV-2 0ΔNLS-immunized mice contained high levels of IgG antibody that bound the many viral proteins present in HSV-2− cells (right panel, Figure 6A). These results suggested that the increased antigenic breadth of the live HSV-2 0ΔNLS vaccine elicited a stronger IgG antibody response against total HSV-2 antigen than a gD vaccine. A two-color flow cytometry test was developed to test this hypothesis by measuring ‘pan-HSV-2 IgG antibody’ levels in the serum of vaccinated mice and guinea pigs (Figure 6B). This quantitative analysis confirmed that a live HSV-2 0ΔNLS vaccine elicited an average 40-fold higher IgG antibody response against total HSV-2 antigen than a gD + alum/MPL vaccine (Table 2 in Ref. [58]).

Western blots were used to determine whether IgG antibodies in serum of HSV-2 0ΔNLS-immunized mice were directed predominantly against gD or whether some of HSV-2’s other 74 proteins might also serve as antigens (Figure 6C & 6D). Serum from gD + alum/MPL-immunized mice contained IgG antibodies that bound an approximately 55 kDa protein expressed by wild-type HSV-1 or HSV-2 (lanes 2 and 4, Figure 6C). This approximately 55 kDa protein was confirmed to be gD as it was not present in cells inoculated with HSV-1 gD− or HSV-2 gD− mutant viruses (lanes 3 and 5, Figure 6C). In contrast, serum from HSV-2 0ΔNLS-immunized mice contained high levels of IgG antibodies against many viral antigens other than gD (outside red box in Figure 6D). IgG antibodies in HSV-2 0ΔNLS-immunized mice predominantly targeted 90–150 kDa viral proteins, which were expressed by both wild-type HSV-2 and a HSV-2 gD− mutant virus (lanes 4 and 5, Figure 6D).

Based on these and other analyses, there appear to be two reasons that a live HSV-2 0ΔNLS vaccine yields 50-fold greater protection against genital herpes than a gD subunit vaccine. First, the live HSV-2 0ΔNLS vaccine stimulates a quantitatively greater adaptive immune response, as evidenced by approximately 40-fold higher levels of pan-HSV-2 IgG antibody [58]. Second, the increased antigenic breadth of the live HSV-2 0ΔNLS vaccine elicits a more polyclonal adaptive immune response, as evidenced by IgG antibodies against >10 viral proteins (Figure 6C & D).

Caveats
If the efficacy of the live HSV-2 0ΔNLS vaccine depends on a polyclonal immune response to HSV-2, then this should be evident both in the T-cell and B-cell responses to the live HSV-2 0ΔNLS vaccine. A major limitation of the dataset presented herein is that my laboratory has yet to formally test the hypothesis that the increased antigenic breadth of the live HSV-2 0ΔNLS vaccine elicits a more polyclonal T-cell response distributed across many HSV-2 proteins. Western blots containing total HSV-2 proteins provide a valuable tool to analyze the immunodominance hierarchy of the IgG antibody response elicited by the live HSV-2 0ΔNLS
Figure 6. Live HSV-2 OΔNLS elicits a greater and far more polyclonal B-cell response to HSV-2 protein antigens than a gD subunit vaccine. (A) Representative immunofluorescent labeling of fixed HSV-2 plaques with a 1:6,000 dilution of mouse serum from a naive mouse, gD-immunized mouse or HSV-2 OΔNLS-immunized mouse. Mouse IgG binding was visualized with AlexaFluor594-labeled goat antimouse IgG (H+L). (B) Two-color flow cytometric analysis of a fixed, single-cell suspension of CFSE-labeled, HSV-2-infected (HSV-2+) Vero cells mixed with uninfected (UI) Vero cells. Fixed cells were incubated with a 1:6,000 dilution of mouse serum from a naive mouse, gD-immunized mouse or HSV-2 OΔNLS-immunized mouse. Mouse IgG binding to HSV-2+ cells was detected using allophycocyanin-labeled goat antimouse IgG, and a flow cytometer to measure CFSE (FL1) and APC (FL4) fluorescent intensity. (C and D) Western blots of proteins from Vero cells that were uninfected (UI) or were infected with 5 pfu per cell of; wild-type HSV-1; HSV-1 gD; wild-type HSV-2 and HSV-2 gD. Western blots were incubated with a 1:20,000 dilution of serum from (C) a gD-2-immunized mouse or (D) a 0ΔNLS-immunized mouse. Red boxes highlight the 55–65 kDa size range of HSV-1’s and HSV-2’s gD protein.

HSV-2: Herpes simplex virus 2; MFI: Mean fluorescent intensity.

vaccine (Figure 6D). While western blot analysis indicates gD is not the most dominant antigen of the HSV-2 0ANLS vaccine, the relative lack of IgG antibody binding to gD in western blots should not be interpreted to mean that the HSV-2 0ANLS vaccine does not elicit a detectable antibody response against gD. Rather, the sensitivity of western blot testing requires animal serum be diluted 1:20,000 to obtain a readable pattern of IgG antibody-labeling of individual proteins (Figure 6D). In contrast, our published gD-antibody capture ELISA data, which demonstrate that 0ANLS-immunized mice and guinea pigs possess gD-antibody titers in the range of 1:20,000–1:100,000 [45,58], were obtained using a 200-fold higher concentration of serum. Moreover, HSV-2 proteins in western blots are denatured, and thus cannot detect IgG antibodies directed against conformational epitopes of gD (Figure 6D).

Finally, with regard to the quantity of ‘pan-HSV-2 IgG antibody’ shown in Figure 6A & 6B, one may postulate that a large portion of this IgG antibody will bind intracellular antigens of fixed cells and would be irrelevant in vivo. However, I note that the magnitude of pan-HSV-2 IgG antibody measured in individual animals (Figure 6B) strongly correlates with the magnitude of functional protection against HSV-2 vaginal challenge in both vaccinated mice and vaccinated guinea pigs [58]. The strength of the observed correlation raises the alternative possibility that IgG antibodies may contribute to vaccine-induced protection against HSV-2 genital infections through mechanisms that we do not yet fully appreciate.

**Antibodies or T-cells: which is the key to an effective HSV-2 vaccine?**

The relevance of humoral versus cell-mediated immunity in vaccine-induced protection against HSV-2 remains incompletely defined. It is evident from decades of studies dating back to Oakes, 1975 [178], that adoptively transferred anti-HSV antibodies or B-cells alone are insufficient to prevent peripheral HSV-1 infection from progressing to fatal disease in immunodeficient nude or severe-combined immunodeficient (SCID) mice [177,179]. In contrast, adoptively transferred T-cells are sufficient to allow such immunodeficient animals to survive peripheral infection with low virulence strains of HSV-1 [177,179]. Moreover, T-cells play a direct role in controlling HSV-1 and HSV-2 infections in sensory ganglia [114,115,168–170,172,180]. Thus, vaccine-induced protection against HSV-2 will almost certainly depend upon the T-cell response to HSV-2 antigens [1,22,49,166,181].

I would suggest that complete, vaccine-induced protection against HSV-2 genital herpes lesions will most likely be dependent upon a balanced B-cell (antibody) and T-cell response to HSV-2’s antigens. Two lines of evidence support this interpretation. First, SCID mice reconstituted with both B- and T-cells control HSV-1 infection significantly more rapidly than SCID mice reconstituted with T-cells alone (Figure 1C in [179]); numerous investigators have reported similar findings with HSV-1 or HSV-2 [182–184]. Second, T-cells alone are slow to infiltrate sites of HSV-1 or HSV-2 challenge unless chemokines [123] or inflammatory stimuli [185] are used to artificially increase the rate of T-cell recruitment. In contrast, antibodies are approximately 100 billion-fold smaller than T-cells and may rapidly enter virus-infected tissues; hence, antibodies may act during the first 24 h to restrict HSV-2 replication and/or spread [58].

Against this background, I would propose that a logical function for anti-HSV-2 antibodies would be to serve as the first line of adaptive immune defense that triggers pro-inflammatory events (e.g., complement cascade), which may help recruit T-cells into virus-infected tissues at the portal of HSV-2 entry (e.g., the vagina). While this hypothesis is consistent with the available evidence, it remains to be formally tested. Therefore, it will be of interest to explore this and other alternative hypotheses that may explain the respective roles of humoral and cellular immunity in vaccine-induced protection against HSV-2.

**Live HSV-2 vaccines: an unexplored opportunity to prevent genital herpes**

Live HSV-2 vaccines offer a new hope for improving HSV-2 vaccine efficacy and ending the ongoing HSV-2 genital herpes epidemic. Before we may explore this opportunity, we must consider the basis of the concerns that raise the possibility that live HSV-2 vaccines may be intrinsically unsafe and thus not suitable for use as human vaccines. There appears to be a growing willingness to reconsider live HSV-2 vaccines for human testing, as a Phase I clinical trial of Sanofi Pasteur’s replication-disabled HSV-2 ACAM-529 vaccine recently commenced [186]. This ACAM-529 vaccine is derivative of 16 years of work from David Knipe, Lynda Morrison and colleagues [46,49,59,120,187,188]. The protracted timeline between development and testing of ACAM-529 in a human clinical trial speaks volumes about the level of suspicion that continues to surround live HSV-2 vaccines.

**Risks of replication-disabled HSV-2 vaccines**

When it comes to replication-disabled HSV-2 vaccines, such as ACAM-529, the absolute risk is immeasurably small. This is because the ACAM-529 vaccine strain bears two deletions in the UL5 and UL29 genes, which are each essential for viral DNA synthesis [120]. In the absence of UL5 or UL29, HSV-2 ACAM-529 cannot complete a single cycle of replication in vaccine recipients. Thus, ACAM-529 is not able to cause disease in SCID hosts [46], nor would it be transmissible between vaccine recipients and their human contacts. Likewise, a replication-disabled HSV-2 vaccine could not conceivably pose a risk to newborns as the capacity of any virus to cause disease will be strictly dependent on its capacity to replicate in vivo.

A bizarre double-standard exists today in how we discuss live-attenuated vaccines used in clinical practice versus genetically engineered, live HSV-2 vaccine candidates that are far safer [43,44,56,189]. Most of the live-attenuated viral vaccines in use around the world (e.g., polio, mumps, measles, rubella, chickenpox and yellow fever) were isolated around the time
Watson and Crick were elucidating the central dogma of molecular biology [190,191]. Although these live viral vaccines are considered ‘safe enough’ to use in humans, genetically they lie a hair’s breadth away from becoming disease-causing viruses. This is because the polio, mumps, measles, rubella, chickenpox and yellow fever vaccines were isolated at the dawn of cell culture methods and rely on random, single nucleotide changes for their attenuated phenotype. There are many point mutations in the larger, live VZV Oka vaccine, but the precise identity of the random nucleotide change(s) that confer the attenuated phenotype remains unclear [53,55,192]. Thus, some of the live viral vaccines in clinical use are genetically metastable and may spontaneously revert and become disease-causing pathogens. This is particularly a problem for the oral polio vaccine; spontaneous reversion explains why live oral poliovirus vaccine frequently reacquires the ability to cause mini-epidemics [193].

Beyond the issue of genetic stability, all live viral vaccines used in clinical practice today are transmissible from vaccine recipients to others. This may lead to severe disease if the live polio, mumps, measles, rubella, chickenpox, shingles or yellow fever vaccines are transmitted to immunocompromised persons.

In contrast, HSV-2 ACAM-529 vaccine is a genetically engineered virus that is missing several kilobases of DNA from the UL5 and UL29 genes [120], is unable to replicate in host cells and is unable to cause disease in severely immunodeficient hosts [46]. Thus, HSV-2 ACAM-529 is far safer than any of the live viral vaccines that we currently administer to millions of children each year. Critics have raised the hypothetical concern, ‘What if a live HSV-2 vaccine strain meets up with wild-type HSV-2 in a vaccine recipient and undergoes recombination?’ This concern is implausible for several reasons. First, if a person is already infected with wild-type HSV-2, then the millions of copies of wild-type HSV-2 in their bodies pose a much greater health risk than the stated concern. Second, recombination between wild-type HSV-2 and a live HSV-2 vaccine strain is technically possible but cannot increase the copy number of viable HSV-2 viruses by even a single copy. This is because DNA recombination is a reciprocal process. If a single copy of live HSV-2 vaccine DNA were to successfully acquire functional viral gene(s) by allelic exchange with a wild-type HSV-2 DNA, then the wild-type HSV-2 would receive attenuated viral gene(s) in exchange and would lose its disease-causing potential. Although DNA recombination between a live HSV-2 vaccine and wild-type HSV-2 is technically possible, there is no logical scenario in which this poses a health risk.

It should be noted that recombination between a live viral vaccine strain and wild-type virus is not unique to HSV-2; this scenario is equally possible in the context of the polio, yellow fever, mumps, measles, rubella and chickenpox vaccines. However, in 50 years of clinical practice, this hypothetical scenario has not proven to be an empirically measurable risk.

Risks of live-attenuated HSV-2 vaccines

An important concern with the replication-disabled HSV-2 ACAM-529 vaccine is the lack of precedent for the approach. If the ACAM-529 vaccine succeeds, it will be the world’s first replication-disabled viral vaccine used in clinical practice. In contrast, the yellow fever, polio, mumps, measles, rubella and chickenpox vaccines provide ample precedent that a live-attenuated virus that undergoes limited recombination in vaccine recipients may be used to evoke protective immunity against a wild-type pathogen. For these reasons, a live-attenuated HSV-2 vaccine offers our best chance of success in defeating genital herpes, but the safety of live-attenuated HSV-2 vaccines is generally considered suspect [49-52]. Below, I consider the basis for such claims.

Historically, there was considerable fear of live-attenuated HSV-2 vaccines because it was believed in the 1970s that HSV-2 was a cancer-causing virus and that a vaccine that delivered HSV-2 DNA into human recipients could pose a cancer risk [194,195]. Forty years later, we know that cervical cancer has a viral co-factor, human papillomavirus [196], and we know that HSV-2 is not linked to cancer. Nonetheless, the seeds of fear and suspicion were planted, and a prevailing belief emerged that a HSV-2 vaccine should be incapable of establishing a latent infection. Replication-disabled HSV-2 vaccines (e.g., ACAM-529) were developed to address such unfounded concerns as a replication-disabled HSV-2 virus cannot establish a latent infection [49-52,189].

The potential of live-attenuated HSV-2 vaccines to establish latent infections in sensory neurons of vaccine recipients has dampened enthusiasm for their testing and development. However, the relevant question that remains unaddressed is, ‘Are the concerns surrounding a live α-herpesvirus vaccine that establishes latent infection serious enough in nature to justify its exclusion from human clinical trials (Figure 3)?’ I present two lines of evidence that these concerns have been blown out of proportion.

First, despite the fact that billions of people are latently infected with wild-type HSV-1 and/or HSV-2, the only known health risks of latent HSV infection are contingent upon HSV’s capacity to reactivate and cause recurrent herpetic disease (i.e., not cancer). Likewise, complications such as neonatal herpes and increased risk of HIV acquisition are downstream consequences of HSV-2 reactivation and/or recurrent genital herpes. All live HSV-2 vaccine approaches discussed to date would be unable to reactivate or cause recurrent herpetic disease [44,48,56,90,189,197]. While other concerns may linger about the live-attenuated HSV-2 vaccine concept, I would suggest that it is time to analyze the veracity of such claims. To date, the concerns surrounding live HSV-2 vaccines have been nebulous in nature and have lacked the type of evidence that typically differentiates science from science-fiction.

The second line of evidence that a live α-herpesvirus vaccine may be acceptable is based on experience with the live VZV Oka vaccine strain. VZV is an α-herpesvirus, which shares 60 homologous genes and a neurotropic-life cycle in common with HSV-2 [53,55,192]. Despite the fact that VZV Oka establishes latent infections in vaccine recipients [55,198], more than 90 million doses of live VZV vaccine have been distributed [54,55]. After 20 years of experience in the USA, the live VZV Oka vaccine has proven to have an excellent safety profile.
and has been effective in reducing VZV-associated disease [54,199]. Accidental carriage of this live α-herpes virus vaccine strain into newborns has not been a major complication [54,200,201]. However, the live VZV Oka vaccine may cause breakthrough disease in immunosuppressed vaccine recipients, such as children with AIDS or cancer [202–205].

In this context, live-attenuated HSV-2 ICP0 mutant viruses should offer significant advantages as they are far more attenuated than the live VZV Oka vaccine. Specifically, live HSV-1 ICP0 and HSV-2 ICP0 mutant viruses are approximately 800-fold more sensitive to repression by the innate-IFN-α/β-response of animal hosts than wild-type virus [56,206–208]. As a result, HSV-1 ICP0 mutant viruses, such as HSV-1 0’GFP and HSV-1 n212, are hypersensitive to repression by the innate IFN response, and thus establish avirulent infections in lymphocyte-deficient SCID mice and rag2-/- mice over a test period of 60–90 days [57]. Moreover, HSV-1 ICP0 mutant viruses are incapable of efficiently reactivating from neuronal latency [57,197,209].

Mutations in the ICP0 gene profoundly attenuate HSV-2, but do not eliminate virulence to the level observed in HSV-1 ICP0 mutant viruses. For example, the ICP0 mutant virus HSV-2 0ΔNLS virus produces lethal disease in 80% of lymphocyte-deficient rag2-/- mice over a 100-day observation period [56]. Likewise, the more attenuated HSV-2 0ΔRING virus, which lacks 40 amino acids of ICP0’s RING finger region, produces lethal disease in 20% of rag2-/- mice over a 100-day observation period [56]. In contrast to IFN-sensitive HSV-2 ICP0 mutant viruses, wild-type HSV-2 strain MS produces uniformly lethal disease within 6–8 days after inoculating immunocompetent mice under the same conditions [56].

Most live-attenuated viral vaccines administered to children have not been evaluated in lymphocyte-deficient SCID or rag2-/- animals. One exception is the live VZV Oka vaccine, which exhibits modest two- to fourfold reductions (relative to wild-type HSV-2) in viral protein synthesis, formation of infectious virus, and production of chickenpox-like lesions in wild-type VZV) in viral protein synthesis, formation of infectious virus, and production of chickenpox-like lesions in wild-type VZV. The risk posed by the live VZV Oka vaccine may pose a health risk to human recipients. Caution is warranted, but the relevant point is that most of the live HSV-2 vaccines proposed to date [43,44,46,48,51,52,56,90,213] are far safer than the live viral vaccines used in clinical practice to prevent mumps, measles, rubella and chickenpox. Logically, we should either stop using live viral vaccines altogether or allow safer live HSV-2 vaccines to advance to human clinical trials; I would recommend the latter option.

The low risks of live HSV-2 vaccine strains should be weighed against the certainty that millions of people will continue to contract, and suffer from, HSV-2 genital herpes for as long as we lack an effective vaccine. In the time that elapses between the writing and publication of this review, five million people will be newly infected with HSV-2. After decades of trying to improve HSV-2 subunit vaccines [3,9,13,28,214], perhaps it is time to consider a different approach. The available evidence indicates that killed or live HSV-2 vaccines represent a safe and feasible means to prevent genital herpes. If we start evaluating killed and/or live HSV-2 vaccines in human clinical trials today, then I predict that in five years we will be ushering in a new era in which the superior efficacy of HSV-2 viral vaccines is undeniable, and vaccine-induced prevention of HSV-2 genital herpes is no longer a goal, but a burgeoning reality.

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Key issues

- Most herpes simplex virus 2 (HSV-2) vaccines proposed to date are subunit vaccines that expose the human immune system to 1% of HSV-2’s antigens. Glycoprotein D is the best studied HSV-2 vaccine, but has failed to elicit robust protection against genital herpes in human clinical trials.
- Live HSV-2 vaccines are a logical alternative, but have not been carefully evaluated in US clinical trials due to a myriad of safety concerns, which are not well supported by evidence.
- Live HSV-2 vaccines appear to be 50 times more effective than gD subunit vaccines in preventing genital herpes in mice and guinea pigs.
- The minimal health risks of vaccinating the human population with a live-attenuated HSV-2 vaccine would be preferable to the unchecked spread of wild-type HSV-2.
- Antigenic breadth may explain why past HSV-2 subunit vaccines have failed, and why live HSV-2 vaccines are more likely to succeed in preventing HSV-2 genital herpes.

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Papers of special note have been highlighted as:
• of interest
•• of considerable interest


• Sixth failed human clinical trial of herpes simplex virus 2 (HSV-2) glycoprotein subunit vaccine; n = 8323 subjects enrolled in this study (of 31,770 screened).


•• Fifth human clinical trial of HSV-2 glycoprotein subunit vaccine; n = 2714 subjects enrolled in this study; equivocal positive results reported, but these results were not reproduced in the sixth clinical trial run from 2003 to 2009 [108].


• Fourth failed human clinical trial of HSV-2 glycoprotein subunit vaccine; n = 2393 subjects enrolled in this study.


• Third failed human clinical trial of HSV-2 glycoprotein subunit vaccine; n = 202 subjects enrolled in this study.


• Second failed human clinical trial of HSV-2 glycoprotein subunit vaccine; n = 98 subjects enrolled in this study.


• First failed human clinical trial of HSV-2 glycoprotein subunit vaccine; n = 161 subjects enrolled in this study.


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