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Herpes Vaccine

Current Status and Future Prospects

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Summary

Recent advances in recombinant DNA technology have greatly expanded the number of approaches to developing safe and effective vaccines, including those for the herpes simplex virus (HSV). The current focus of clinical evaluation is on HSV subunit glycoprotein vaccines, although it is uncertain if these represent the optimal immunogens for inclusion in vaccines or whether the addition of other proteins would enhance efficacy. Similarly, the optimal adjuvants for formulation with subunit vaccines have yet to be defined. Other approaches that allow viral proteins to be synthesised within cells offer certain advantages and could lead to the vaccines of choice, although many questions remain regarding these strategies.

Herpes simplex virus (HSV) infections are extremely common throughout the world. Advances in serological methods can now distinguish HSV type 1 (HSV-1) from type 2 (HSV-2) infections. Studies using these methods have shown that HSV-1 infections can be documented in 40 to 95% of adults and HSV-2 infections in <10 to 95% of adults. Infection rates depend on cultural factors, socioeconomic level and sexual practices.[1]

Historically, HSV-1 infections have been associated with disease above the waist, while HSV-2 infections were considered to be sexually transmitted and cause disease below the waist. However, each virus can produce indistinguishable disease at either site, and each is associated with a high rate of asymptomatic and unrecognised infections. Both viruses establish a life-long persistent (latent) infection in sensory ganglia which can reactivate to produce symptomatic or asymptomatic recurrent infection.

Although antiviral therapy can ameliorate primary and recurrent infections and prevent recurrences when taken prophylactically, no therapy has been shown to reduce or eliminate the persistent viral infection. The most cost-effective and efficient strategy for control of HSV disease is the development of safe and effective vaccines.

1. Vaccine Goals

The first trial of an HSV vaccine was conducted more than 70 years ago, yet the development of an effective vaccine remains elusive. Immunisation could affect HSV disease at 3 points:

- a prophylactic vaccine might interfere with viral replication at the entry site, e.g. the skin or mucous membrane, thus altering the progress of acute primary infection
- a prophylactic vaccine could also reduce or prevent viral entry into or replication in the peripheral nervous system, thus preventing or reducing viral latency
- a therapeutic vaccine might induce or enhance

immune responses, thus reducing recurrent HSV disease.

Each goal is associated with difficulties. The optimal vaccine for prophylaxis would not only reduce disease, but would also reduce infection (viral replication) and most importantly prevent or reduce the establishment of latency. In keeping with these goals, the Committee on Issues and Priorities for New Vaccine Development of the National Academy of Sciences of the USA has suggested that a successful HSV vaccine should provide:[2]

- 50% reduction in the number of symptomatic primary infections
- 75% reduction in the number of recurrences
- approximately 60% reduction in the severity of disease.

From a public health viewpoint, HSV vaccines should not only reduce symptomatic disease, but should also reduce viral shedding from asymptomatically infected individuals. Both symptomatic and asymptomatic viral shedding are associated with transmission of HSV infection.

2. Approaches to Vaccine Development

Viral vaccines, including HSV vaccines, have historically been divided into live or killed vaccines. Recombinant DNA technology has extended the possible approaches to vaccine development to include subunit vaccines, genetically engineered viral and bacterial vectors and nucleic acid–based vaccines. In this article, we briefly consider the current status of vaccine development and future directions. Other reviews on this subject have appeared recently.[3-6]

2.1 Live Virus Vaccines

The use of live viral vaccines has certain advantages over killed or subunit vaccines with regard to the induction of immune responses, but also certain disadvantages related largely to problems of safety. Approaches to immunisation with live virus include:

- use of live viral vectors such as vaccinia to express selected HSV proteins
- live avirulent HSV vaccines
- replication-defective viruses.

2.1.1 Live Vectors Expressing Viral Glycoproteins

Several laboratories have demonstrated protective efficacy against primary infection in animal models using vaccinia and adenovirus vectors expressing HSV glycoproteins.[7-10] Although vaccinia is a potentially useful vector, there are concerns about its virulence and its ability to induce an immune response in vaccinia-seropositive individuals. More recently, this approach has been extended to replication-defective vectors in the poxvirus family, including fowlpox and canarypox.[11]

Adenovirus vectors have also shown utility in animal models. The ability to administer adenovirus vaccines orally or nasally could have an advantage by inducing mucosal responses.[12] These approaches have not yet been evaluated in human trials of HSV vaccine.

2.1.2 Live Avirulent Vaccines

Another vaccine strategy is based on the development of live avirulent HSV vaccines. Animal experimentation has established that prior infection with viral mutants protects animals against challenge with wild-type virus.[13-15] However, concerns remain regarding the oncogenic potential, degree of attenuation and ability of the live virus vaccines to revert (by recombination, complementation or otherwise) to wild-type virus.

Recently, stable viral mutants have been genetically engineered by inactivating or deleting genes involved in neurotropism, neurovirulence, latency or reactivation.[16] These viruses have shown efficacy in various animal models including *Aotus* monkeys,[17,18] and 1 candidate vaccine (R-7020) has been tested in small phase I clinical trials.[19] To our knowledge, however, no further trials are planned with this vaccine because of poor immunogenicity. Alternative constructs are, however, being developed that will replicate to a

higher titre,[6] including a γ 34.5 deletion mutant.[20]

2.1.3 Replication-Incompetent Viruses

The most recent variation in live virus vaccines is the use of replication-incompetent viruses.[21-24] These contain a mutation in an essential gene and are propagated in a cell line that can actively express that gene. When administered as a vaccine, these viruses can only complete a single cycle of replication and, even if they establish a latent infection, they cannot reactivate to produce infectious virus. Initial experiments have demonstrated the utility of this approach in animal models, but this strategy has not been tested in human trials.

2.2 Killed Virus Vaccines

The traditional approach to immunisation utilises inactivated vaccines containing crude or purified proteins derived from virus-infected cell cultures. For HSV, the shortcomings of this approach include:

- difficulties in producing consistent concentrations of the important immunogens
- the problem of assuring complete inactivation and elimination of contaminating viral DNA that may potentially be oncogenic
- the costs involved.

Clinical studies demonstrated that an HSV-2 glycoprotein vaccine prepared from virus-infected fibroblasts was immunogenic when administered in sufficient amounts.[25,26] Unfortunately, a large efficacy trial[27] used a low dose of vaccine that was poorly immunogenic; hence, the efficacy of the vaccine could not be established. Consequently, the future of this vaccine is uncertain.

Similarly, a purified glycoprotein D vaccine prepared from infected cells appeared safe and immunogenic in preliminary trials,[28] but the vaccine has not been evaluated further.

2.3 Genetically Engineered Subunit Vaccines

The use of virus-infected cells to produce immunogenic viral proteins has largely been supplanted by the development of genetically engineered subunit vaccines composed of defined viral polypeptides. This approach overcomes the 3 disadvantages listed in section 2.2 for traditional inactivated vaccines.

The initial strategy involved expression of HSV-1 glycoprotein D in *Escherichia coli*.[29] This strategy was improved upon by the insertion of truncated viral genes into mammalian expression vectors engineered so that the sequence data coding for the anchor portion of the protein was deleted. In this way, mammalian cells synthesise a glycosylated HSV glycoprotein that is secreted into the medium.

Preclinical evaluations have largely focused on glycoproteins B and D. Studies have shown that these subunit vaccines reduce not only primary disease and viral replication, but also subsequent recurrences.[30-32] Many different adjuvants, including cytokines such as interleukin-2[33] and a fusion protein of glycoprotein D and interleukin-2[34] have been evaluated.

The first clinical trials of a genetically engineered HSV-2 glycoprotein D vaccine are under way. Initial reports[35] indicate that the vaccine elicits few adverse effects. Four doses of 30 to 100μg of glycoprotein D adsorbed to alum induced antibody responses to glycoprotein D in HSV-seronegative individuals approaching or exceeding those detected in patients with genital herpes. Neutralising antibody and cell-mediated immunity (lymphoproliferative responses) were also induced in the majority of vaccine recipients. This is viewed as a distinct improvement over previous vaccine candidates.

Trials using similar vaccine preparations from another manufacturer[36] are due to begin shortly.

2.4 Genetic Immunisation

The newest innovation in vaccine development is genetic immunisation, whereby DNA or RNA encoding a protein is injected directly into the animal. The nucleic acid is apparently taken up by cells and processed to produce an immunogenic protein. Because the protein is being made inside

the cell, this approach offers some of the benefits of live vector immunisation without the concerns for attenuation.

Preliminary work using DNA encoding a specific influenza protein demonstrated the ability to induce CD8+ cytotoxic T cells and protection against influenza.[37] This work is being extended to HSV in several laboratories.

3. Immunotherapy

The idea of stimulating immune responses in the latently infected host to modify recurrent HSV disease is not new. Initial approaches included autoinoculation of patients with their own HSV isolate at a second site,[38] and nonspecific stimulation of the immune system.[39] More recently, this strategy has been extended to include use of inactivated and subunit vaccines.

Because of poor experimental design, especially the lack of control groups, most clinical trials of immunotherapy have yielded uninterpretable results. However, 2 carefully controlled studies[40,41] failed to support the concept of immunotherapy, although it was unclear if these trials failed because the concept of immunotherapy is invalid or because the vaccines were poorly immunogenic.

Recent studies using an animal model of recurrent genital herpes provided the first controlled experimental evidence that administration of immunogenic HSV glycoproteins with potent adjuvants can significantly reduce the frequency and severity of recurrent HSV infection.[42-44] These experiments suggest that augmentation of specific cell-mediated immune responses will be critical in developing successful immunotherapeutic vaccines.[45] Animal studies using the adjuvants muramyl dipeptide, muramyl tripeptide and monophosphoryl lipid A, and the immunomodulator imiquimod, have further demonstrated the utility of this approach with adjuvants that may be accepted for use in humans.[43,46]

The recent success of immunotherapy using the HSV-2 glycoprotein D/alum vaccine in patients with frequent recurrences is especially exciting. Straus et al.[47] reported that 2 immunisations with 100μg of glycoprotein D in an alum vehicle reduced the rate of HSV genital recurrences from 0.55/month to 0.42/month ($p = 0.055$), with a decrease in the median number of recurrences from 6 to 4 per patient over the 1-year period of observation ($p = 0.039$). The decrease in recurrences was greatest during the first 4 months of the study. It is likely that vaccines containing adjuvants that are more able to induce cell-mediated immune responses will improve upon the modest efficacy demonstrated in this early trial.

4. Conclusions

Efforts to develop an effective HSV vaccine are progressing well along several different pathways. New insights into the molecular biology of the virus have identified candidate proteins for inclusion in subunit vaccines, and are being used for the rational development of live attenuated vaccine candidates. The use of genetic immunisation and replication-incompetent viruses offers other unique strategies that may combine the best of subunit and live virus vaccines. Further, for the first time, we have evidence that vaccines can not only be applied as prophylaxis but also as therapeutics for the millions of individuals who develop recurrent HSV disease.

There is, however, much more to be learned that will contribute to improving vaccines. Our understanding of mucosal immunity, especially as it applies to sexually transmitted diseases, is poor. We need to better understand the role of local antibodies and cell-mediated immune functions that prevent or limit infection at this portal of entry in order to develop effective strategies for prophylactic vaccines. A better understanding of the immunology of recurrent HSV disease is also required to improve the rational development of therapeutic vaccines. We need to continue our efforts to define the target antigens to be included in vaccines, the immune correlates for protection, and adjuvants or other strategies to induce them.

The future of HSV vaccine development appears bright. We have learned from the mistakes of

past vaccine trials that suffered from poor design and/or poorly immunogenic vaccine candidates. Today, there are several ongoing clinical trials of subunit vaccines that include proteins identified as protective in animal trials and adjuvants that should provide protection. These trials are placebo-controlled and double-blind, using highly exposed populations and designed to provide the statistical power to identify protection in these individuals. We are anxiously awaiting the results of these trials and excited about evaluating new strategies now in development.

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