

# Clinical Trials of Prophylactic and Therapeutic Herpes Simplex Virus Vaccines

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## KEY WORDS

■ HSV-2 ■ GENITAL HERPES ■ PROPHYLACTIC VACCINE ■ THERAPEUTIC VACCINE ■ ATTENUATED LIVE VACCINE ■ SUBUNIT VACCINE ■ DISABLED INFECTIOUS SINGLE CYCLE VACCINE ■ EFFECTIVE IMMUNITY ■ TRANSMISSION ■ STI

## SUMMARY

Herpes simplex virus type 2 (HSV-2) is a suitable target for a vaccine, despite available antiviral therapies, because the virus causes lifelong infection and significant medical and psychosocial morbidity. A vaccine has the potential to reduce HSV acquisition, disease severity and the number of cases of neonatal herpes. It could also reduce transmission of HIV, which is epidemiologically linked to HSV. Prophylactic vaccines for HSV-2 must give broad and durable immunity across all mucosal surfaces to be effective. This is a significant challenge, as the major determinants of effective immunity have not yet been identified. Even if full protection cannot be achieved, vaccines would still be useful if they could increase the threshold of infection, or prevent clinical disease. However, it is possible that a vaccine could reduce symptomatic disease, but not eliminate asymptomatic shedding, which could inadvertently increase transmission from individuals who believe they are not infectious. Investigated prophylactic vaccines for HSV-2, including subunit vaccines encoding HSV glycoproteins packaged with adjuvants, have shown some benefits. The Chiron gD2gB2-MF59 vaccine gave transient protection of less than 6 months. The GlaxoSmithKline gD2-alum MPL vaccine conferred a 73–74% reduction in acquisition of symptomatic HSV-2 disease and a 38–42% reduction in the acquisition of HSV-2 infection in HSV-seronegative women, but gave no protection in men or HSV-1 seropositive women. Therapeutic vaccines aim to prevent HSV recurrences or minimise disease severity and duration, thereby reducing transmission. Research indicates that to be effective, therapeutic vaccines need to stimulate strong cell-mediated immune responses. Vaccines have induced HSV-specific antibody responses alone but have failed to protect recipients from recurrences. Further research is needed to define determinants of immunity to HSV-2, including identifying HSV-2 antigens, in order to design more effective vaccines.

## Introduction

GENITAL HERPES IS a cause of medical and psychosexual morbidity among many individuals. As herpes simplex virus type 2 (HSV-2) infection is lifelong and because of its continued burden, even with the availability of effective antiviral therapy, HSV is a suitable candidate for a vaccine. The benefits of a vaccine range from those that affect the individual (e.g. reduction in the acquisition of genital HSV infection or reduction in the severity of disease; fewer cases of neonatal herpes) to those at a population level (e.g.

reducing the epidemic spread of herpes; reducing the risk of HIV acquisition/transmission).

Vaccines for HSV were first studied in the 1920s<sup>1</sup> and in the intervening eight decades there have been many attempts to develop one: the approaches employed range from live vaccines through to subunit vaccines developed using molecular biology (Table 1). These have been used prophylactically or therapeutically for recurrent HSV infection. This paper discusses the varying degrees of success with each type of vaccine and considers future options.

**Table 1: Types of vaccines that have undergone clinical evaluation**

|  |
|--|
| Autoinoculation of live HSV                            |
| Whole inactivated vaccines                             |
| Attenuated live virus vaccines                         |
| Modified live virus subunit vaccines                   |
| Cell culture-derived subunit vaccines                  |
| Recombinant subunit vaccines (glycoprotein vaccines)   |
| Disabled infectious single cycle (DISC) virus vaccines |
| Nucleic acid (DNA) vaccines                            |

## Prophylactic HSV-2 Vaccines

### EXPECTATIONS FOR THE EFFICACY AND SAFETY OF A PROPHYLACTIC HSV-2 VACCINE

The ideal objective of a prophylactic vaccine is to induce sterilizing immunity, which must not only be broad and durable but must also be effective at all portals of HSV entry (e.g. genital mucosa, facial mucosa and the eye). A vaccine that provides effective immunity against HSV must produce an immune response much more powerful than the response produced with natural infection. If such immunity could be achieved, then the virus would be eliminated before it could cause acute HSV disease and establish latency, thereby preventing both primary disease and recurrences. Sterilizing immunity represents a significant challenge, as HSV replication occurs in the genital tract of animals re-inoculated with virus after first-episode genital HSV infection,<sup>2,3</sup> and the major determinants of effective immunity have not successfully been identified. However, even if sterilizing immunity cannot be achieved with first-generation vaccines, a vaccine could be beneficial if it was able to:

- Shift the threshold of infection (i.e. the titre of virus necessary to give a primary infection and establish latency);
- Prevent clinical manifestations of the infection (i.e. prevent clinical disease).

If a vaccine could confer a partial protective immune

## RECOMMENDATIONS AND STATEMENTS

- Research on the cellular and mucosal responses to HSV-2 infection is required to identify determinants of effective immunity (research need recommendation)
- Research should be conducted to identify HSV-2 antigens that will generate effective immunity (research need recommendation)
- No randomized clinical trials have to date demonstrated a clinically useful benefit from a therapeutic vaccine for HSV-1 or HSV-2 infection. Further candidate vaccines and studies are required in order to develop effective therapeutic vaccination strategies (research need recommendation)
- Recent studies suggest some potential benefit of a prophylactic vaccine containing gD2 and MPL among HSV-seronegative women. Further work in

response then, theoretically, an immunized individual would require a larger inoculum of virus to become infected. Shifting the threshold of infection may result in the prevention of recurrences in a proportion of vaccinees, clinically milder symptomatic disease in some, or asymptomatic infection in others. A caveat is that if a vaccine does not completely prevent latency, HSV will continue to reactivate and the majority of transmission occurs during asymptomatic shedding.<sup>4</sup> As a result, vaccine recipients would still be at risk of intermittently shedding virus, although the titres might be lower, which may reduce the risk of infecting others. A vaccine that prevents symptoms but does not alter the long-term natural history of reactivation, or has no effect on asymptomatic shedding, may be detrimental. The outcome could be an increase in the reservoir of people with undiagnosed infection, which would lead to higher rates of transmission. Thus, it is uncertain whether a partially protective vaccine alone would reduce or increase the spread of infection, particularly in the absence of universal immunization. However, when used with other interventions that do not completely interrupt virus transmission, even a partially protective vaccine may help reduce the risk of HSV transmission by rendering infected vaccinees less infectious and by making those who were uninfected less susceptible to infection.

### ATTENUATED LIVE VIRUS VACCINES

Attenuated live virus vaccines that cause a primary infection but do not reactivate after establishing latency in the ganglia should stimulate an immune response. The attenuated vaccine should not reactivate from latency, even in a severely immunocompromised patient. The stability of attenuation is important, as genetic recombination could otherwise occur with wild-type virus.

R7020 is the only attenuated live HSV vaccine tested in clinical trials to date.<sup>5</sup> It was constructed from an HSV-1 strain by deleting a portion of the thymidine kinase gene and by replacing the sequences representing the internal inverted repeats and adjacent genes in the L component with a fragment of the HSV-2 genome encoding the glycoproteins (g) G, D, I, and a portion of E. The gD sequence was added because of its immunogenicity and the gG sequence was inserted to act as a marker of successful immunization.<sup>5</sup> An HSV-1 DNA fragment encoding the thymidine kinase gene fused to the  $\alpha 4$  gene promoter was added back into the construct.

The modified virus was stable and established latency at a reduced rate in mice, guinea pigs and rabbit models.<sup>5</sup> In the same models, R7020 protected against wild-type virus challenge.<sup>5</sup> However, the vaccine was poorly immunogenic in initial clinical studies and serial administration of  $10^5$  plaque forming unit (pfu) doses of the candidate vaccine was required to elicit an immune

improving its effectiveness is needed (research need recommendation)

## RECOMMENDATION AND STATEMENT CATEGORIES

### Category 1

Consistent evidence from controlled clinical trials. For example, for an antiviral, this would include results from at least one well-designed, randomized, controlled clinical trial, and, in the case of laboratory studies, consistent evidence from comparative studies.

### Category 2

Evidence from at least one well-designed clinical trial without randomization, from cohort or case-controlled analytical studies (preferably from more than one centre), or from multiple time-series studies or dramatic results from uncontrolled experiments.

### Category 3

Evidence from opinions of respected authorities based on clinical experience, descriptive studies or reports of expert committees.

### Research Need

Area in which research is warranted.

response. At this very high dose, R7020 was poorly tolerated in HSV-1 seropositive individuals, in whom it caused adverse events severe enough to stop the trial.<sup>6</sup>

Although the R7020 live attenuated vaccine was unsuccessful, should a more immunogenic construct be developed, this option offers an excellent immunological approach as it presents a broad range of HSV antigens to the host's immune system.

## SUBUNIT VACCINES

- Research on the cellular and mucosal responses to HSV-2 infection is required to identify determinants of effective immunity (research need recommendation)
- Research should be conducted to identify HSV-2 antigens that will generate effective immunity (research need recommendation)

### Glycoproteins as targets for immune responses: A

recent generation of vaccines centred on one or two glycoproteins, which were given in combination with adjuvants to boost their immunogenicity. These vaccines were produced by recombinant DNA technology, which allows production of large quantities of glycoprotein free of whole virus or HSV DNA.

There are 11 glycoproteins in HSV, which are inserted into the envelope of the virus and perform important biological functions (Figure 1). There is little strain differentiation in the major surface proteins of HSV, in contrast to the persistent RNA viruses which use antigenic change as a major mechanism for their persistence.<sup>7</sup> For example, gD is a highly conserved protein with more than 98% identity among strains.<sup>8</sup> In addition gD DNA shows no sequence changes when sequenced over several reactivation episodes.<sup>9</sup>

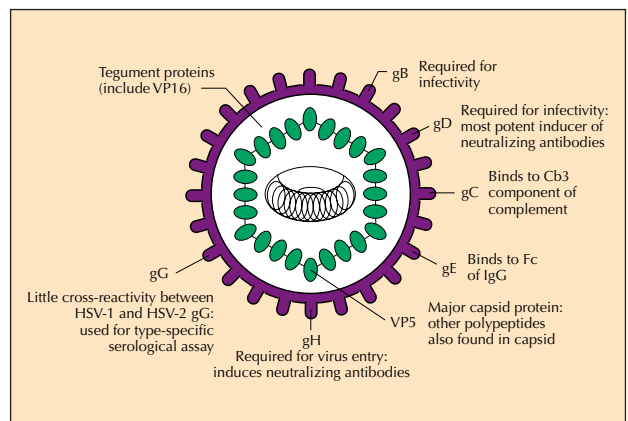


Figure 1:  
Structure of HSV.

Importantly, gD is a confirmed target of neutralizing antibody, antibody-dependent cellular cytotoxic responses and of virus-specific immune responses mediated by CD4+ and CD8+ cells.<sup>10</sup> Thus, if glycoproteins prove to stimulate protective responses, their conservation avoids the need for strain-specific vaccines.

Other glycoproteins which trigger cellular and humoral immune response include gB, gC and gE.<sup>10</sup> Neutralizing antibodies to HSV are predominantly directed against gB and gD and monoclonal antibodies to these glycoproteins protect guinea pigs and mice from experimental challenge with HSV.<sup>11–13</sup> Therefore, stimulating immune responses to gB and gD offers a potential strategy for protecting against infection with HSV or against HSV reactivation. However, a disadvantage of using only a few glycoproteins is that the immune response generated is limited to a few epitopes and a full array of cellular immune responses is probably not developed.<sup>14</sup>

**Chiron gD2gB2-MF59 vaccine:** A prophylactic HSV-2 vaccine, developed by Chiron, that comprised truncated gD2 and gB2 (glycoproteins from HSV-2) with the adjuvant MF59, was ineffective in two randomized, double-blind, placebo-controlled studies.<sup>15</sup> The first study of the gD2gB2-MF59 vaccine enrolled 531 HSV-2 seronegative partners of HSV-2-infected persons, and the second study included 1862 individuals attending sexually transmitted infection (STI) clinics who were at high risk of HSV-2 infection. In both trials, the volunteers were given the vaccine or placebo intramuscularly at 0 months, 1 month and 6 months and followed for up to 1 year after the third immunization.<sup>15</sup> The primary outcome measure was acquisition of HSV-2 infection as assessed by seroconversion to HSV-2 antigens not contained in the vaccine (i.e. antigens other than gD2 and gB2).

During the initial 5 months of the studies, the acquisition rate of HSV-2 among vaccine recipients was 50% lower than among placebo recipients. However, the effect was transient and by the end of the one-year follow-up period the overall efficacy was 9%. Interestingly, in a *post hoc* analysis there appeared to be a difference in efficacy by gender with an efficacy rate ( $[\text{HSV-2 acquisition rate in vaccine group} - \text{rate in placebo group}] / \text{HSV-2 acquisition rate in placebo group}$ ) of 26% (95% confidence interval [CI], -29%, 58%) in women compared with 4% (95% CI, -64%, 34%) in men. In both studies, the vaccine failed to reduce the likelihood or severity of symptomatic disease.

The reasons why gD2gB2-MF59 vaccine provided only transient protection against HSV-2 infection, despite inducing high titres of HSV-2 specific antibody, are unclear. Behavioural factors, such as the use of condoms or number of sexual exposures, did not differ between the study groups.<sup>15</sup> Hypothetical explanations for the loss of efficacy include a short-lived protective immune response (the precise composition of which is unknown), or the possibility that initial protection offered by the vaccine was lost with frequent exposure to HSV-2. Because of the failure to show a prolonged effect on the acquisition of HSV-2 infection or on the clinical course of the disease, work on the vaccine was halted.

Based on results from maternal–fetal transmission studies,<sup>16–18</sup> it has been hypothesized that there is a threshold of HSV-2 specific antibody above which protection against disease is evident.<sup>15</sup> However, the results from both gD2gB2-MF59 vaccine trials showed no differences in peak HSV-2 antibody titres following vaccination between ‘vaccinated, uninfected’ individuals and those who were vaccinated but went on to become infected.<sup>15</sup> These results suggest that neutralizing antibody alone is not sufficient to protect against genital HSV-2 infection.<sup>19</sup> This vaccine apparently failed to elicit a sufficiently broad range of

effector responses. In two HSV-2 antibody-dependent cellular cytotoxicity (ADCC) assays, there were relatively low antibody responses, especially among HSV-seronegative individuals, leading to consideration that low ADCC responses contributed to the reduced efficacy of the gD2gB2-MF59 vaccine.<sup>20</sup> It is also possible that the vaccine failed to induce critical cell-mediated immune responses important in preventing infection with HSV-2. This suggests more research on the cellular and mucosal responses to HSV-2 infection is required before the determinants of effective immunity can be identified. Once the correlates of protection are ascertained, the objective of vaccine design should be to enhance these responses.

#### **gD2-Alum MPL vaccine:**

● Recent studies suggest some potential benefit of a prophylactic vaccine containing gD2 and MPL among HSV-seronegative women. Further work in improving its effectiveness is needed (research need recommendation)

A vaccine developed by GlaxoSmithKline, containing truncated gD2 and alum combined with a potent adjuvant (3-de-O-acylated monophosphoryl lipid A; MPL) was well tolerated and induced humoral and cellular immune responses superior to gD2 and alum alone in a Phase I trial.<sup>21</sup> The vaccine was subsequently assessed in two double-blind, randomized, placebo-controlled trials.<sup>22</sup> In one study (Study 1), 847 HSV-1- and HSV-2-seronegative partners (268 women) of HSV-2-infected individuals were enrolled from 57 centres. In the other study (Study 2), 2491 partners (of any serostatus) of HSV-2 infected persons were enrolled from 61 centres. Of them, 1867 were negative for HSV-2 antibodies, 710 of whom were HSV-2-seronegative women; 554 were negative for both HSV-1 and -2 antibodies, 200 of whom were HSV-seronegative women. The vaccine was administered intramuscularly at 0 months, 1 month and 6 months. The duration of the study was 19 months, with the main end-point being protection against HSV-2 disease. Source partners could not use suppressive antiviral therapy in the first study but were allowed to do so in the second study.

Overall, in both studies, the protection against HSV-2 disease offered by the vaccine and control were similar: the vaccine's efficacy was 38% in HSV-1- and HSV-2-seronegative men and women in Study 1, and 42% in HSV-2-seronegative female subjects in Study 2. The gD2-alum-MPL subunit vaccine was 73–74% effective in preventing symptomatic genital HSV-2 disease in women who had no pre-existing antibody to HSV-1 or HSV-2 ( $P=0.01–0.02$ ). The trend to reduction in HSV infection was not significant, even among women who were HSV-1 and HSV-2 seronegative (46% in Study 1; 39% in Study 2). There was no evidence that the vaccine was protective in women who were initially HSV-1-seropositive or in men. Confirming the results of the Phase I trial, the vaccine was safe, generally well tolerated, and induced gD-specific neutralizing antibodies and a T helper (Th)1 cell-mediated immune profile.<sup>22</sup>

The selection of adjuvant was a major difference in the formulations of the Chiron and GlaxoSmithKline vaccines. The Chiron vaccine contained MF59 while the GlaxoSmithKline vaccine contained alum and MPL. The partial effectiveness of this glycoprotein vaccine compared with the failure of the Chiron glycoprotein vaccine suggests that adjuvant may be critical in facilitating the induction of protective immune responses. More research is needed to understand the adjuvant effects as well as the unexpected gender-specific protection seen with the GlaxoSmithKline vaccine.

These results provide the most promising evidence yet of the efficacy of a vaccine to prevent genital herpes. A further National Institutes of Health/GlaxoSmithKline-

**Table 2: Results of early double-blind human trials of inactivated virion vaccines for therapeutic prevention of HSV infection<sup>31</sup>**

| Author (year)                                 | Vaccine preparation               | Therapeutic trials Improved/Total |    |         |    |
|---|-----------------------------------|-----------------------------------|----|---------|----|
|   |                                   | Vaccine                           |    | Placebo |    |
|   |                                   | n                                 | %  | n       | %  |
| Kern and Schiff (1964) <sup>38</sup>          | Whole virus, formalin-inactivated | 16/23                             | 70 | 22/29   | 76 |
| Weitgasser <i>et al.</i> (1977) <sup>41</sup> | Whole virus, heat-killed          | 28/34                             | 82 | 18/60   | 30 |
| Kutinova <i>et al.</i> (1988) <sup>44</sup>   | Subunit, lectin-purified          | 18/24                             | 43 | 15/42   | 35 |

sponsored, double-blind, randomized, controlled trial is underway. The primary purpose of this study is to assess the prophylactic efficacy and safety of the vaccine in the prevention of genital herpes disease in 7550 women who are HSV-1 and -2 seronegative. The volunteers are randomized to receive either the HSV-2 vaccine or an investigational formulation of a hepatitis A vaccine at 0 months, 1 month and 6 months. The study will last for approximately 20 months.

## Therapeutic HSV-2 Vaccines

### EXPECTATIONS FOR THE EFFICACY AND SAFETY OF A THERAPEUTIC HSV-2 VACCINE

- No randomized clinical trials have to date demonstrated a clinically useful benefit from a therapeutic vaccine for HSV-1 or HSV-2 infection. Further candidate vaccines and studies are required in order to develop effective therapeutic vaccination strategies (research need recommendation)

The objective of a therapeutic vaccine is to prevent recurrences or at least minimize their severity and duration. In doing so, a therapeutic vaccine should decrease contagiousness by either preventing or limiting virus reactivation in the ganglion, or by minimizing virus replication after egression from the nerve and before transport to mucosal surfaces. To achieve this, a vaccine must augment a host's specific immune responses, but this goal is challenging: immune responses to 'natural' primary infection with HSV do not protect against virus reactivation. As the mechanisms that control outbreaks are different to those required to prevent initial infection,<sup>23,24</sup> a therapeutic vaccine will likely have to boost different immune responses to that of a prophylactic vaccine.

The role of a cellular immune response in humans is suggested by the lack of severe or more frequent herpes recurrences in agammaglobulinaemic patients, which contrasts with the more severe disease pattern seen among people with AIDS and those who have undergone cytotoxic chemotherapy or organ transplantation (i.e. those with defects in cell-mediated immunity).

T-cells play an important part in HSV-specific immunity. CD4+ cells predominate in the early stages of genital HSV lesions and are followed by CD8+ cells.<sup>23,24</sup> These two cell types act to resolve HSV lesions. CD4+ cells are the main source of the cytokine interferon- $\gamma$ , which appears to be important in the control of HSV recurrences.<sup>25,26</sup> In patients with frequent recurrences of orofacial herpes, the titres of HSV-specific antibody were higher and there was a trend for lower levels of HSV-specific interferon- $\gamma$  and interleukin-2 production by peripheral blood mononuclear cells.<sup>27</sup> Interferon- $\gamma$  stimulates the expression of major histocompatibility (MHC) class II antigens in cells and allows CD4+ lymphocyte cytotoxicity. This cytokine also partially reverses the HSV-induced downregulation of MHC class I expression, enabling CD8+ lymphocytes to lyse infected cells. Depletion experiments in animal models suggest that the protection against genital HSV-2 re-infection is mediated more by CD4+ cells and

interferon- $\gamma$  than by CD8+ cells or antibody.<sup>28-30</sup>

It is likely that for a therapeutic HSV vaccine to be effective, it must stimulate virus-specific cellular immunity. The challenge in developing an effective therapeutic vaccine is to identify the HSV antigen(s) that induce(s) a greater protective response than infection with whole virus. If this could be achieved, the theoretical goals would be to:

- Eradicate latent infection;
- Prevent recurrent disease;
- Prevent asymptomatic shedding.

The benefits of achieving any of these objectives with a therapeutic vaccine are the same as for a prophylactic vaccine but, by the same token, many of the same caveats apply. For example, a vaccine that prevents symptomatic recurrences but does not influence asymptomatic shedding could potentially enhance the transmission of the virus. A therapeutic vaccine that does not eradicate latent infection but decreases shedding may be useful in limiting the risk of vertical transmission for pregnant women with a history of recurrent genital herpes. Therapeutic vaccines may be an alternative to antiviral therapy, particularly for individuals who are intolerant to antiviral drugs or whose infections are refractory to these agents. A vaccine would also offer a more convenient regimen to antiviral therapy, even if booster doses of the vaccine were required. A limitation of therapeutic vaccines is that they would not be expected to be of use for the management of HSV infections in immuno-compromised patients.

Several approaches have been employed for the immunotherapy of HSV infection (Table 2).<sup>31</sup> Many of the earlier trials of candidate vaccines had significant methodological flaws that render their interpretation difficult, but the newer vaccines have undergone rigorous clinical trials.

### NON-SPECIFIC LIVE VACCINES

Non-specific live vaccines were first used in the 1920s when smallpox (vaccinia) vaccines were used in open clinical trials.<sup>1</sup> The theoretical basis for this approach was that HSV and vaccinia stimulate cross-protective immunity, an assumption that was later disproved.<sup>32</sup> The bacillus Calmette-Guerin (BCG) was also used as a non-specific immune stimulant but failed to show efficacy in a double-blind placebo-controlled trial.<sup>33</sup> The mean rate of recurrence over 9 months of prospective follow-up was 0.528 recurrences per month in BCG recipients compared with 0.392 recurrences per month in placebo recipients. BCG also failed to influence the duration of lesions in the first recurrent episode of genital herpes after vaccination. Moreover, persistent cutaneous granulomas were noted in three of these six patients given a second inoculation.

### AUTOINOCULATION OF LIVE HSV

In the 1950s and 1960s, individuals were inoculated with their own vesicular fluid in an attempt to treat recurrences. This challenge did not prevent further episodes of HSV reactivation and lesions often occurred at the injection site as well as at the sites of the original recurrences.<sup>34,35</sup>

**Table 3: Effect of therapeutic vaccination with Lupidon G and Lupidon H vaccines on genital herpes<sup>40</sup>**

| Parameters                 | Vaccine group<br>(n=142) | Control group<br>(n=50) | P-value |
|----------------------------|--------------------------|-------------------------|---------|
| Recurrence (number/year)   | 2.92 ± 2.95              | 6.22 ± 2.05             | <0.001  |
| Disease-free time (days)   | 205.64 ± 135.92          | 61.58 ± 25.38           | <0.001  |
| Recurrence duration (days) | 2.85 ± 2.62              | 4.78 ± 1.98             | <0.001  |
| Active disease (days/year) | 11.59 ± 15.3             | 30.4 ± 17.49            | <0.001  |

Adapted with permission from *Int J STD AIDS* 1995;**6**:431–435.

**WHOLE, INACTIVATED VIRION VACCINES**

The safety concerns accompanying live vaccines prompted the development of inactivated virus preparations, prepared by chemical or physical treatment of the virus. This type of vaccine has the disadvantage that it induces less broad and less durable immune responses than do live-virus preparations, but this shortcoming can be overcome by using an adjuvant.

In the 1930s, Frank performed the first clinical trial of an inactivated HSV vaccine for the treatment of recurrent HSV infection.<sup>36</sup> The vaccine was a suspension of formalin-inactivated, HSV-infected rabbit brain. It was repeatedly injected into 14 volunteers, 13 of whom reported subjective lengthening of the interval between recurrences. A placebo control group was not included, but despite this, the study design by Frank was the model for a large number of inactivated virus vaccine studies conducted up until 1982. In these studies, up to 100% of patients reported improvements in recurrent HSV disease but the assessments of efficacy were subjective. For example, a formalin-inactivated vaccine given to over 2000 individuals provided subjective improvements for 65–80% of patients.<sup>37</sup>

Kern and Schiff<sup>38</sup> conducted the first placebo-controlled studies of an inactivated whole virus vaccine in the early 1960s (Table 2). In this study, in which participants were followed for 6–36 months, 16 of 23 vaccine recipients (70%) had fewer recurrences but 22 of 29 placebo recipients (76%) also experienced improvements.<sup>38</sup> The significant placebo effect in the second study underscores the importance of appropriate controls when evaluating a vaccine for the immunotherapy of HSV infection.

**Lupidon G and H vaccines:** Heat-killed, whole virus vaccines from HSV-2 (Lupidon G) and HSV-1 (Lupidon H) produced significant improvement in 81% (858/1059) of participants in an uncontrolled, open trial.<sup>39</sup> This report of success led to the two Lupidon vaccines being investigated in double-blind, placebo-controlled trials (Tables 2 and 3).<sup>40,41</sup>

The subjects in the Lupidon vaccines study were recruited from nine Italian university clinics.<sup>40</sup> Importantly, the individuals were not differentiated according to whether they had facial or genital herpes: in the vaccine group, 69 had facial herpes only, 65 had genital herpes only and eight individuals had both diseases; in the control group, 24 had facial herpes and 26 had genital herpes. These diseases have distinct natural histories<sup>42</sup> and should not be combined for analysis.

The volunteers were randomized to receive both vaccines or aciclovir (at variable doses of 1200–2000 mg/day until resolution of an episode). An aggressive inoculation regimen was followed: 1 ml of the vaccine preparation was administered subcutaneously in the deltoid region every week for Months 1–3, every 2 weeks for Months 4–5, and monthly at Months 6–9 and Month 12. The methods used to evaluate the outcome were not stated in the publication of the trial.<sup>40</sup> The follow-up period was up until Month 15 after the last vaccine dose. The Lupidon vaccines had a statistically significant effect on recurrences of genital and facial herpes (Table 3<sup>40</sup>). Although these differences were significant, the study had several weaknesses:

there was a diverse study population; many treatments were given; and there was a failure both to report the method of assignment to treatment group and to define the method of outbreak assessment.

There are many disadvantages of inactivated virion vaccines, including difficulties in ensuring that all virus is inactivated.

**INACTIVATED, SUBUNIT VACCINES**

Inactivated HSV subunit vaccines contain components of the virus rather than the whole virus. They are produced by various techniques including detergent extraction, DNase treatment and affinity chromatography. These processes have the following disadvantages: they are labour intensive and costly, the yield is often low, and the product is difficult to reproduce and standardize. Despite these drawbacks, there have been several clinical trials of subunit vaccines for HSV immunotherapy.<sup>43,44</sup>

As with the whole inactivated vaccines, design flaws hamper the interpretation of many of these clinical studies. For example, in a non-blinded study of a subunit vaccine containing HSV-2 envelope glycoproteins and alum, there were fewer and less frequent recurrences in 59 volunteers receiving the vaccine than in 33 control subjects.<sup>43</sup> When recurrences did occur in the vaccine recipients, they were of shorter duration and less severe. Unfortunately, a placebo effect may have confounded the results, as the control group knew it was not receiving the vaccine. In addition, the study group comprised individuals with genital HSV infection and those with orofacial herpes.

Methodological issues accompanied a trial of an HSV-1 glycoprotein vaccine absorbed to alum for the treatment of recurrent genital or facial herpes.<sup>44</sup> There was a significant placebo effect, with similar decreases in the frequency of recurrences in the vaccine recipients (43%) and in those who received placebo (35%). Furthermore, the vaccine was minimally immunogenic, eliciting little or no humoral response.

**Skinner vaccine:** The NFU.Ac.HSV-1(S-)MRC (Skinner) vaccine consists of 300 mg of mixed HSV-1 glycoproteins prepared by formalin inactivation and detergent extractions. Prophylactic and therapeutic efficacy is claimed from a series of uncontrolled studies.<sup>45,46</sup> The Skinner vaccine was also assessed for the therapy of genital HSV infection in a double-blind, placebo-controlled trial in 316 patients with frequently recurring genital herpes.<sup>47</sup> The vaccine was administered at 0 months, 1 month and 2 months (Table 4). In comparison with placebo, the vaccine significantly reduced the number of recurrences per month in women at 3 months and 6 months after the third dose.

Only male vaccinees had fewer lesions per recurrence but their healing time was unaffected. Overall, the vaccine recipients had an increased HSV-1 neutralizing antibody titre compared with placebo at 1 month following the last vaccination ( $P=0.002$ ) and an increased lymphocyte proliferation response to HSV-1 but not HSV-2. This increase in lymphocyte transformation response was restricted to male vaccine recipients and was only statistically significant when

**Table 4: Therapeutic trial of the NFU.Ac.HSV-1(S-)MRC (Skinner) vaccine<sup>47</sup>**

|                                   | Males   |         | Females |         | Both    |         |
|-----------------------------------|---------|---------|---------|---------|---------|---------|
|                                   | Placebo | Vaccine | Placebo | Vaccine | Placebo | Vaccine |
| <b>Recurrences per month (n)</b>  |         |         |         |         |         |         |
| Median                            | 0.6     | 0.6     | 0.7     | 0.5*    | 0.7     | 0.6     |
| Range                             | 0–1.6   | 0–2.6   | 0–4.2   | 0–1.9   | 0–4.2   | 0–2.6   |
| (subjects)                        | (62)    | (66)    | (86)    | (84)    | (144)   | (148)   |
| <b>Lesions per recurrence (n)</b> |         |         |         |         |         |         |
| Median                            | 1.3     | 1.0*    | 1.3     | 1.3     | 1.3     | 1.2*    |
| Range                             | 1–3     | 1–3.9   | 1–6     | 1–9.6   | 1–6     | 1–3.7   |
| (subjects)                        | (56)    | (59)    | (79)    | (78)    | (135)   | (137)   |

\*P<0.05. Reproduced with permission from Skinner GR, Turyk ME, Benson CA *et al.* The efficacy and safety of Skinner herpes simplex vaccine towards modulation of herpes genitalis; report of a prospective double-blind, placebo-controlled trial. *Med Microbiol Immunol (Berl)* 1997;**186**:31–36. © 1997 Springer-Verlag.

compared with pre-vaccination levels, not when assessed against placebo. Therefore, the Skinner vaccine showed no consistent pattern of efficacy or immunogenicity.<sup>47</sup>

**CLINICAL TRIALS OF RECOMBINANT GLYCOPROTEIN VACCINES**

**gD2-alum vaccine:** The first recombinant glycoprotein vaccine to undergo clinical trials for immunotherapy of HSV infection consisted of 20 µg of truncated gD2 vaccine adsorbed onto alum. This increased HSV-specific antibody titre in a Phase I trial to a greater extent than any previous vaccine.<sup>48</sup> Twenty-four individuals with and without prior HSV-1 or -2 infection were inoculated four times with 30 µg or 100 µg of gD2 in alum. The peak enzyme immunoassay (EIA) antibody titres in seronegative individuals were higher than the initial titres in seropositive individuals. The HSV-2 seropositive volunteers had 6–10-fold increases in their EIA antibody titres and 6–7-fold increases in neutralizing antibody titres.

The success of the gD2-alum vaccine in this preliminary trial led to a double-blind, placebo-controlled trial in 98 patients with recurrent genital herpes.<sup>49</sup> These individuals had between 4 and 14 recurrences per year. They were injected with 100 µg gD2 in alum or a control of alum alone at entry into the study and at 2 months. The patients were followed for a year and their recurrences were confirmed clinically and by culture. By several criteria, the gD2-alum vaccine recipients experienced fewer genital herpes recurrences than subjects given placebo. The gD2 recipients reported 24% fewer recurrences per month than the control group (0.42 versus 0.55; P=0.055) and 36% fewer culture-proven recurrences per month (0.18 versus 0.28; P=0.019). Over the year of the study, there was a lower median number of total recurrences in the vaccinees (4 versus 6; P=0.039) (Figure 2). The vaccine also increased neutralizing antibody titres four-fold and gD2-specific EIA titres seven-fold.

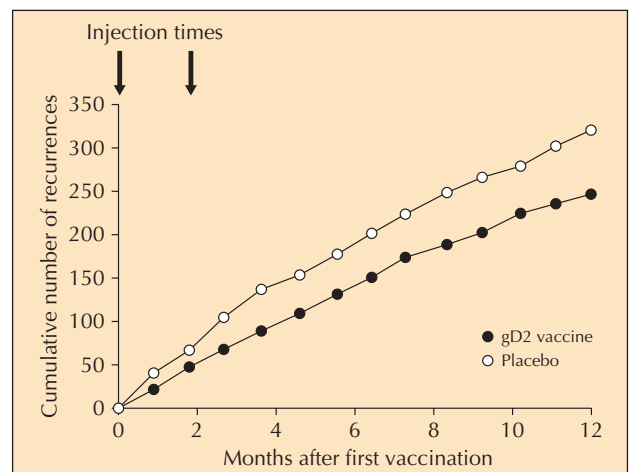
**gD2gB2-MF59 vaccine:** The gD2-alum vaccine was the first vaccine to modify the course of a chronic viral disease in a placebo-controlled clinical trial. However, the alum used in the vaccine is not a potent (immunogenic) adjuvant. In an attempt to improve upon the vaccine, gD2 was combined with other adjuvants and with other glycoproteins. The adjuvant MF59 was known to boost immunogenicity in guinea pigs<sup>50</sup> with reactogenicity that was higher than that of alum but judged to be acceptable. Animal models had demonstrated that the combination of gB2 and gD2 was immunogenic and effective in the therapy of HSV infection.<sup>51</sup> Therefore, a vaccine combining gB2 and gD2 with MF59 was evaluated clinically. Following dose-ranging studies, the vaccine formulation tested for

therapeutic efficacy contained lower antigen doses (10 µg each of gB2 and gD2 in MF59 versus 100 µg of gD2 in alum) given the greater potency of the combination with MF59 adjuvant as measured by peak antibody titres.

The peak antibody titres in HSV-2 seronegative individuals following vaccination with the adjuvant were higher than those with the gD2-alum vaccine, and the mean neutralizing antibody titre was higher than in individuals with naturally acquired HSV-2 infection. There were also sustained increases in T-cell responses with the gD2gB2-MF59 vaccine that were not observed with the gD2-alum vaccine.<sup>10</sup>

Following on from the promising initial studies, the gD2gB2-MF59 vaccine was entered into a double-blind, placebo-controlled therapeutic study in 202 individuals with recurrent genital herpes.<sup>52</sup> Half of the participants received vaccine (gD2gB2-MF59) at 0 months, 2 months and 12 months, and placebo with MF59 only at 14 months. The other half received placebo at 0 months and 2 months and vaccine at 12 months and 14 months. The participants were followed for 18 months, during which recurrences, cellular and humoral responses were assessed.

The vaccine did not significantly reduce the monthly rate of genital HSV recurrences by either culture or clinical assessment (Table 5). In addition, the duration of virus shedding was not significantly reduced by the vaccine.<sup>52</sup> Other disease parameters were significantly reduced during vaccination, including symptom duration, new lesion formation and lesion duration. However, the lack of effect on recurrence rate led the manufacturer to stop trials of the vaccine as therapy for



**Figure 2:** Genital herpes recurrences in 98 infected individuals vaccinated with gD2-alum or alum alone.<sup>49</sup> Reprinted with permission from Elsevier (*Lancet* 1994;**343**: 1460–1463).

**Table 5: Monthly recurrences in a placebo-controlled trial of gD2gB2-MF59 adjuvant vaccine<sup>52</sup>**

| Mean monthly rate of recurrences | Vaccine | Placebo | P-value |
|----------------------------------|---------|---------|---------|
| Culture positive recurrences     | 0.2     | 0.2     | NS      |
| Clinically confirmed recurrences | 0.4     | 0.4     | NS      |
| All lesional episodes            | 0.4     | 0.6     | NS      |
| Duration of virus shedding       | 2.7     | 3.8     | NS      |
| Duration of symptoms             | 2.7     | 6.7     | 0.002   |
| New lesion formation             | 4.1     | 6.9     | 0.04    |
| Duration of lesions              | 6.8     | 8.8     | 0.004   |

NS, not significant.

HSV infection.

The effect of the gD2gB2-MF59 vaccine on recurrence rate was lower than that with the gD2-alum vaccine.<sup>49</sup> The reasons for this difference may include a potentially suboptimal antigen dose (10 µg each of gD2 and gB2 in MF59 versus 100 µg of gD2 in alum). This hypothesis is supported by the observation that the HSV-2 specific antibody responses with the gD2gB2-MF59 vaccine did not increase to the same extent as with the gD2-alum vaccine. However, as noted when the gD2gB2-MF59 vaccine was used prophylactically, it is possible that the humoral response does not correlate with protection. It is also possible that MF59 did not facilitate induction of the cell-mediated immune responses that are important in controlling recurrent HSV infections. The outcome of this trial highlights the importance of antigen and adjuvant selection and dose in the design of therapeutic HSV vaccines.

#### DISABLED INFECTIOUS SINGLE CYCLE (DISC) VIRUS VACCINES

Disabled infectious single cycle (DISC) virus HSV vaccines are derived from a virus with an essential gene deleted.<sup>53</sup> When the modified virus infects a host cell, the replication cycle of the virus proceeds to a point where the missing gene product is required. If this is late in the cycle the virus will have most of its components, but no infectious virus is produced. After inoculation, because the DISC virus has the majority of its structural proteins, an immune response will be generated against all of these proteins rather than the selected antigens that are used in subunit and glycoprotein vaccines. Another important property is that the antigens produced after the single cycle of virus replication can be presented in conjunction with MHC class I molecules and stimulate a broad cell-mediated response to virus infection. These attributes make DISC vaccines attractive vaccine candidates.

A DISC vaccine (TA-HSV-2) comprising an HSV-2 strain with gH deleted, has undergone clinical trials. The protein encoded by the gH gene is required for cell entry (Figure 3).<sup>54</sup> To allow the vaccine to infect cells on initial inoculation, the vaccine is propagated in cells that supply the missing gene product. When injected into a host, the modified virus infects a cell and completes a single cycle of replication that results in the assembly and release of fully formed but non-infectious progeny virions.<sup>55</sup>

The TA-HSV-2 vaccine (10<sup>7</sup> pfu dose) was immunogenic and protective in guinea pigs challenged with HSV-2.<sup>53,56,57</sup> Following these preclinical studies, the vaccine underwent clinical trials and was found to be well tolerated and immunogenic among people who have not been previously infected with HSV. However, a Phase II clinical efficacy trial with TA-HSV was unsuccessful. The study was multi-centre, placebo-controlled and conducted among 483 HSV-2 seropositive patients with more than six recurrences of genital herpes per year. There were three treatment arms in which the subjects received 10<sup>7</sup> pfu/dose or placebo

at: 0 weeks and 8 weeks; 0 weeks, 4 weeks and 8 weeks; and at 0 weeks, 2 weeks, 4 weeks and 8 weeks. No significant difference was seen between any treatment arm and placebo with respect to time to first recurrence, the mean number of recurrences or any other clinical outcomes assessed during the course of the study. It was anticipated that the vaccine would reduce asymptomatic virus shedding but there was no difference in this parameter for any immunization regimen and placebo.

#### Conclusions

To date, well-controlled trials have established the potential utility of both prophylactic and therapeutic HSV vaccines. The gD2-alum-MPL vaccine is entering further testing and may be the first prophylactic vaccine for genital herpes, although its use is likely to be restricted to HSV-1-seronegative women. While many of the HSV therapeutic vaccines have not been successful, the gD2-alum vaccine provides a proof of principle that therapeutic vaccines can impact on the natural history of genital herpes. The Lupidon vaccine trial raises the possibility that a more aggressive treatment regimen is needed to stimulate protective immunity. Thus, further candidate vaccines and studies are required in order to develop effective therapeutic vaccination strategies.

The available evidence suggests that cell mediated immunity, rather than humoral responses alone, are important in protecting against genital HSV infection and recurrences. Thus far, most vaccines tested in humans produce large rises in HSV-2-specific antibody titres but this immunological change has not translated to protection. Improved knowledge of the key determinants of protection will be important for the future design of more effective vaccines.

Another area worthy of investigation is the route of

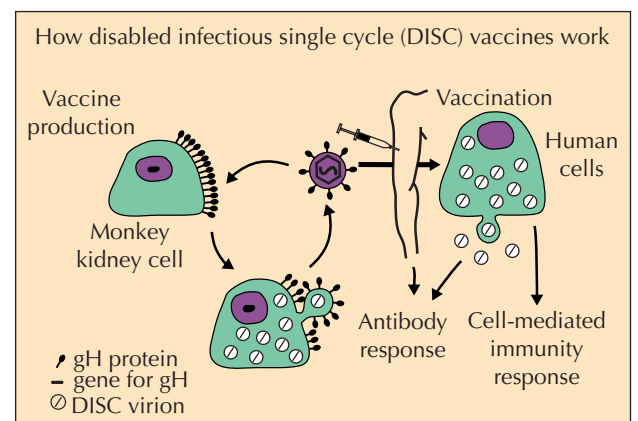


Figure 3: Principle of a DISC vaccine.<sup>54</sup> Reprinted with permission from Elsevier Science (Lancet 1996;**348**:573).

administration of vaccines. In trials of both prophylactic and therapeutic vaccines, they were administered intramuscularly or subcutaneously. However, studies in mice have shown that long-term immunity and protection against HSV-2 results from mucosal inoculation rather than systemic immunization.<sup>58</sup>

Other vaccines are in various stages of commercial development and include:

- Live, genetically-attenuated vaccines (Medimmune, AuRx);
- Prophylactic HSV DISC vaccine (DISC PRO) in development for genital and facial herpes (Xenova);
- Nucleic acid-based vaccines, in which DNA is inoculated into muscle tissue and there is protein expression of the encoded gene (Wyeth-Lederle, PowderJect, Merck). Initial Phase I studies are being conducted.

Both therapeutic and prophylactic vaccines offer the potential of reducing the spread of genital herpes. In

assessing their impact on the epidemic, it will be necessary to understand their effects on asymptomatic shedding. Given that there is currently no effective vaccine available, further research and vaccine development are required. Such efforts should incorporate wide-ranging assessments of the natural history of genital herpes.

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