BioDrugs 1998 Oct; 10 (4): 257-264
1173-8804/98/0010-0257/$04.00/0

Herpesvirus Vaccines
An Update

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Abstract

The development of vaccines against the herpesviruses has major public health importance because of the wide spectrum of associated clinical disease with this virus in both immunocompetent and immunocompromised populations. Because these viruses establish latent infections capable of subsequent reactivation, both immunotherapeutic and prophylactic vaccine strategies are needed. A range of vaccine formulations has been devised, largely as a result of the rapid growth in knowledge in molecular microbiology and genetic engineering, including live and inactivated whole virus vaccines, and subunit vaccines consisting of recombinant viral glycoproteins in various adjuvants. The live attenuated virus Oka strain vaccine is now licensed for prophylaxis against varicella (VZV) in some countries.

Recent studies with herpes simplex viruses (HSV) have demonstrated immunogenicity with glycoprotein vaccines; however, these studies have also highlighted their failure to reduce seroconversion to HSV-2 in high-risk populations. Nevertheless, his work has helped develop comprehensive methodologies for the clinical amd immunological assessment of newer vaccine formulations which have proved successful in animal models. The live attenuated Towne strain vaccine has been shown to prevent severe human cytomegalovirus (CMV) infection in transplant recipients. More recently, subunit antigens and virus vector vaccines against CMV and Epstein Barr virus (EBV) have been devised. Novel attempts to present viral antigens now include the development of plasmid expression vectors for viral nucleic acids and genes as well as engineered live vectors for viral antigens. These new technologies may allow future vaccines to be devised, not only against the 5 well characterised herpes viruses, but also against the recently recognised herpes viruses 6, 7 and 8 whose full clinical spectrum is still unknown.

1. Background

There are currently 8 human herpesviruses (HHV1 to 8) whose disease spectrum ranges from subclinical to life-threatening infections and neoplastic disease. Three of these viruses: HHV6, HHV7 and HHV8, have been identified and characterised relatively recently, whilst the herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2), the varicella zoster virus (VZV or HHV3), the Epstein Barr virus (EBV or HHV4) and the human cytomegalovirus (CMV or HHV5) have been studied over a much longer time period. The greater knowledge of these latter agents and understanding of

their pathogenesis in the human host has allowed development of vaccination strategies for their control.

In attempting to evolve realistic strategies for vaccination against herpesviruses, knowledge of the capability of these viruses to establish latent and reactivating infections[1,2] represents major challenges. For each herpesvirus infection, two approaches to vaccination, therapeutic and prophylactic, can be envisaged. A therapeutic vaccine will promote responses that reduce or ameliorate the duration, frequency and severity of recurrent episodes, whilst a prophylactic vaccine will stimulate immune responses that provide protection against primary infection and, ideally, the establishment of latency. These goals presuppose knowledge of those immune and non-immune factors central to the control of primary and reactivating herpesvirus infections. Although certain elements operating at the mucosal surface, and affecting the elimination of herpesvirus-infected cells are known to be important,[3-6] there is still an incomplete understanding of the mechanisms involved.

There have been advances however, partly through an empirical approach, such that a live, attenuated vaccine developed at the Biken Institute in Japan in the 1970s to prevent disseminated varicella in leukaemic children[7] is now commercially produced and licensed for prophylactic use.[8,9] In addition, vaccines against HSV infections, particularly genital herpes, are currently in clinical trials.[10] Several different vaccine formulations (table I) have been evaluated, including inactivated whole virus vaccines,[11] specific glycoprotein vaccines[13-15] and attenuated live virus vaccines.[12] Assessment of the efficacy of these preparations has been either through their clinical or serological responses, but has more recently been assessed by measurement of cellular parameters of immunity.

2. Recent Developments in Herpesvirus Vaccines

The recent burgeoning of molecular and genetic engineering technology, the development and experimental evaluation of novel adjuvants aimed at enhancing or targeting specific immune responses, and the availability of well characterised animal models for HSV infections has led to intensive development and pre-clinical investigation of new vaccine formulations, with progress in some instances, sufficient to permit clinical trials. These advances have centred particularly around recombinant glycoprotein vaccines either adjuvanted or designed for delivery in some form of vector or fusion protein;[16,17,20-23,27,34-37] genetically engineered vaccines able to undergo only limited replication in the host;[28-33] or DNA vaccines.[24-26]

Table I. Human herpes simplex virus (HSV) vaccine preparations and their evaluation[a]

Vaccine preparation	Substrate or vector	Vaccine evaluation	Reference
Inactivated whole virus	Animal tissues, eggs or cell cultures	Serological; clinical	11
Subunit/DNA-free	Cell culture lysates, envelope glycoproteins	Pre-clinical; serological; clinical	10
Live, attenuated recombinant	Cell cultures	Pre-clinical; serological; clinical	12
Adjuvanted recombinant glycoprotein	Eukaryotic and prokaryotic cell systems	Pre-clinical; serological; lymphoproliferation; CTL; clinical	13-18 19
Vectored glycoprotein	Adenovirus; VZV; Salmonella	Pre-clinical; serological; DTH	20-23
DNA/Plasmid	Nil	Pre-clinical; serological; lymphoproliferation	24-26
Fusion protein	IL-2	Pre-clinical; serological	27
Replication-defective viruses	Genetically-engineered cell cultures	Pre-clinical; serological; lymphoproliferation; DTH, CTL; clinical	28-33

a For further information on types of HSV vaccines, readers are referred to recent review articles[2,10]

CTL = cytotoxic T lymphocytes; **DTH** = delayed type hypersensitivity; **IL-2** = interleukin-2.

There is evidence of a dichotomy in these newer approaches to immunisation with regard to the relative value of a live (attenuated or disabled) replicating viral vaccine, as compared to one based on nonreplicating antigens. Several factors impinge on these different strategies. The route of immunisation to be employed, and the exposure and stimulation of the immune system to either a whole or merely a part of the full range of antigenic components of the virus, may influence the nature and efficacy of the immune response.[4,5,18,38,39] A possible disadvantage of immunisation with a limited number of virion components in vectored glycoprotein antigens or nucleic acid vaccines is that any positive effects on the immune response of the integrated structure of the virus particle or its replicative process will be lost. Whilst the presence and functioning of some viral-coded proteins may be undesirable or unnecessary,[40] or the immunogenicity of these subunit vaccines may be increased by enlightened use of adjuvants, it is possible that an intact, replicating virus or one with some limited replicative ability may more effectively stimulate the required immune responses. However, there are concerns about the risks of live vaccines, such as the possibility of recombination with wild-type virus, and the theoretical oncogenic potential of some herpesviruses.

Current perceptions of immunity against herpesviruses, based largely on studies with HSV or VZV in experimental animals, are that the presence or stimulation of cytotoxic T lymphocytes (CTLs) at the sites of primary or recurrent infection, usually a mucosal surface, may represent an important facet in herpesvirus control.[4,23,41] Recently, it was reported that the level of both CD8 and CD4 T-cell responses generated by immunisation of mice with a replication deficient HSV-1 was sufficient, following adoptive transfer, to limit cutaneous HSV infection, indicating that a complete viral replication cycle was not essential to elicit durable and potent HSV-specific immune responses and protection.[32]

3. Therapeutic Strategies

3.1 Herpes Simplex Viruses

Much consideration has been given to the concept of immunotherapy against recurrent HSV-1 and HSV-2 infections,[42] based on the knowledge that the immune responses of the latently-infected host can be boosted following immunisation with HSV vaccine preparations. A recombinant glycoprotein HSV-2 glycoprotein D (gD) alum-adjuvanted vaccine, was reported in 1993 to significantly boost the gD-2 enzyme-linked immunosorbent assay (ELISA) antibody levels and HSV-2 neutralising antibodies in HSV-seropositive vaccine recipients.[19] In 1994, the same group reported that vaccination of seropositive humans with 2 doses of this preparation was associated with reduced HSV-2 recurrence rates and an increase in the time to first post-vaccination recurrence, compared to a control group given placebo.[13]

More recently, a glycoprotein HSV-2 glycoprotein B (gB) and gD vaccine mixed with the MF59 oil-in-water adjuvant emulsion formulation which includes the metabolisable oil squalene and the surfactants Tween 80 and Span 85, was given in 4 doses to otherwise healthy individuals experiencing 4 to 14 recurrences per year.[15] This preparation, although promoting gB-2 and gD-2 specific antibodies, and HSV-2 neutralising antibodies, reduced the severity of the first post-vaccination genital recurrence but did not reduce recurrence frequency over an 18-month period. In addition, reflecting earlier studies with this adjuvant in HSV-2 seronegatives,[14] there were transient mild to moderate local or systemic reactions in most volunteers.[15]

In 1996, the disabled, infectious, single cycle (DISC) vaccine, lacking the gene for the essential gH glycoprotein, [29,30,39] entered clinical trials in HSV-2 seropositive individuals. Preliminary indications are that this vaccine is well tolerated, and immunogenic in humans, and detailed information with respect to both humoral and cellular immune responses are due later this year.

3.2 Varicella-Zoster Virus

The success of the live, attenuated VZV (Oka strain) vaccine used prophylactically and the reduced rate of zoster in leukaemic children and adults to whom prophylactic Oka vaccine has been administered,[6] suggests that a therapeutic VZV vaccine is a viable goal. The serious morbidity of zoster in individuals with iatrogenic or natural immunosuppression, including the elderly, indicates there is a need for vaccine development in this area. Clinical studies using the Oka-VZV vaccine have been found to boost immunity in adults after 2 doses,[43,44] while killed Oka-VZV has been used in attempts to boost the immunity of the elderly and prevent or ameliorate zoster.[45]

3.3 Other Herpesviruses

In most healthy individuals, reactivation of endogenous CMV, EBV, and the more recently discovered herpesviruses causes no or mild illness. However, in immunosuppressed individuals, such as patients with AIDS or individuals undergoing transplantation surgery, disease caused by reactivation of EBV, CMV or HHV-8 can result in serious morbidity and mortality. Vaccination prior to immunosuppressive therapy in transplant recipients to prevent EBV or CMV reactivation by boosting levels of any pre-existing immunity would be of benefit. However, most of the recent work and clinical trials with vaccines against these herpesviruses, have been undertaken to prevent primary infection.

4. Prophylactic Strategies

4.1 Herpes Simplex Viruses

A vaccine strategy for the prevention of HSV infection and the disease of primary genital herpes is both important and appealing. Recent seroepidemiological studies have shown that genital HSV-2 infections are increasing in many developed countries.[46-48] Although most infections are subclinical and unrecognised, a large burden of ill-health is associated with symptomatic disease. Moreover, genital herpes facilitates HIV transmission,[47,49] and the development of a vaccine to prevent HSV infections is of considerable public health importance.

A plethora of evidence from studies in experimental animal models has demonstrated the potential for a variety of HSV-2 vaccine formulations to induce both humoral and cellular immune responses and protect against HSV challenge.[18,22,23-25,28-30,39] These encouraging results have led in recent years, to placebo-controlled, double-blind clinical trials. In a study in the US, the DISC HSV-2 vaccine has recently been assessed in known seronegative volunteers, and indications are that the vaccine is safe and immunogenic in this population (C.S. McLean, personal communication).

Trials of recombinant HSV-2 gD and/or gB vaccines have been undertaken in HSV-2 seronegative volunteers. In 1995, promising results of a trial of an M59-adjuvanted HSV-2 gD/gB vaccine developed by Chiron Biocine were reported.[14] This preparation, given in 3 doses by the intramuscular route, promoted levels of gD-2 and gB-2 ELISA antibodies and HSV-2 neutralising antibodies equal to or higher than those observed following natural infection. Increases in the frequency of precursor gD-2 and gB-2 specific T-cells were also detected and maintained for 5 months following the second immunisation. Although generally well tolerated, the vaccine did produce some mild, transient local and systemic reactions in some volunteers.

Preliminary results from 2 recent international phase 3 prophylactic trials however, indicate failure of an adjuvanted subunit glycoprotein G2 (gG2) vaccine to prevent seroconversions amongst high-risk seronegative individuals attending sexually-transmitted disease clinics despite excellent tolerability and induction of primary immune responses.[50] Whilst this result is disappointing, the study has helped establish protocols for future rigorous assessment of other HSV vaccines.

In many developed European countries with declining rates of oro-labial HSV-1 acquisition in childhood, up to 50% of primary genital herpes in-

fections are now caused by HSV-1. In view of this, assessment of the degree of cross-immunity to HSV-1 afforded by novel HSV-2 vaccines will merit additional consideration.

4.2 Varicella-Zoster Virus

Whilst the live Oka-VZV vaccine may theoretically establish a latent infection and reactivate or revert to virulence, the Oka-VZV preparation in practice appears to be safely attenuated, possibly because of changes in capsid structure and assembly.[51] The vaccine induces both T-cell and antiviral antibody responses,[52,53] and lasting immune responses have been demonstrated in children.[54] Vaccine adverse effects occur in 15% of children, but these are generally mild consisting of minor skin rash, transient fever and local reactions.[53] In adults, 2 doses of Oka-VZV vaccine are generally required to produce the same degree of cell-mediated immunity and seroconversion as are obtained after a single dose in children.[6]

Recently, the Oka-VZV vaccine has been licensed for universal immunisation of children aged 15 to 18 months in the US and has been shown to elicit a satisfactory immune response in combination with measles-mumps-rubella (MMR) vaccine,[55,56] but whether such immunity is maintained remains to be determined. In Europe, the vaccine has been recommended for selective use in those in whom varicella complications are more common, such as children with leukaemia, susceptible women of child-bearing age, and healthcare workers.

4.3 Other Herpesviruses

A prophylactic CMV vaccine would be most cost-effective if administered to susceptible women of child-bearing age, the immunocompromised and organ-transplant recipients. Primary CMV disease in pregnancy is a major cause of congenital abnormality.[57,58] The live, attenuated Towne strain vaccine was developed nearly 30 years ago[59] and in double-bind, placebo-controlled studies in renal transplant recipients[60-63] has been found to prevent severe CMV disease and induce both humoral and cell-mediated immune responses. The Towne strain does not induce latency,[64] has no demonstrable immunosuppressive effects, and has not been reported to be oncogenic.

Recent work with both CMV and EBV has centred on the development of viral subunit antigens and virus vector vaccines. The CMV gB glycoprotein and the intermediate early proteins IE1 and IE2, which promote strong CD4 and CD8 T-cell responses and anti-viral neutralising antibodies, are likely candidate antigens.[65-67] The occurrence of EBV-associated tumours such as nasopharyngeal carcinoma and Burkitt's lymphoma is indicative of the need to develop EBV vaccines, with young children in equatorial Africa being a prime target group. Recent studies in humans using a licensed vaccinia virus expressing the major EBV envelope glycoprotein gp340 have reported some protection against, or delay in onset of, EBV infection.[37] Work is also in progress on the development of a subunit EBV vaccine based on the CTL epitope from the latent gene for B-cell lymphomas.[68,69]

5. Novel Strategies and Vaccination Against Herpesviruses

The use of viral nucleic acids or viral genes is an exciting new concept for immunisation against viral and other infections,[25,26,69] and plasmid expression vectors encoding HSV glycoproteins B and D have been constructed and assessed in animals.[24,25] A plasmid expression vector containing the entire HSV gD open reading frame when injected into mice, was found to elicit immunoglobulin (Ig) G, but not IgA, antibody responses in both serum and vaginal secretions, to induce cellular immune responses in splenocytes, and to reduce levels of HSV-2 replication in the mouse genital tract.[24] Using the female guinea-pig model of genital herpes, other workers found a plasmid expression vector encoding HSV-2 gB and gD to promote serum ELISA and neutralising antibodies against HSV-2 and to provide significant protection against virus challenge.[25] A plasmid DNA vaccine encoding the gB glycoprotein of HSV-1

and delivered intranasally has also been found to stimulate mucosal IgA in both the genital and enteric tracts of mice.[26] The availability of such vaccines for evaluation or use in humans must, however, await further investigations.

Another intriguing strategy for vaccination against HSV infections is the use of vectors expressing HSV glycoproteins. A recombinant Oka vaccine strain of VZV, into which the HSV-2 gD gene has been inserted, has already been found to result in the development of neutralising antibodies and significant protection against HSV-2 challenge in guinea-pigs.[22] Interestingly, the reverse scenario, the delivery and expression of genes for VZV glycoprotein E and an immediate early protein in a recombinant HSV-1 virus vector is also undergoing investigation.[70]

6. Conclusions

Following the application of modern technology, vaccination of humans against at least some of the herpesvirus infections is becoming a reality. It remains to be seen, however, which type of vaccines will eventually gain widespread acceptance and/or whether different vaccine formulations will be required for immunotherapeutic and prophylactic use. This will undoubtedly depend on the level, breadth and appropriateness of the immunity they promote. The ongoing extensive work into development and evaluation of herpesvirus vaccines may serve not only to provide acceptable and efficacious vaccine preparations but also to provide a greater understanding of the immune responses relevant to the control of these complex infections.

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