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VIROLOGY (A NICOLA, SECTION EDITOR)

Herpes Simplex Vaccines: Prospects of Live-Attenuated HSV Vaccines to Combat Genital and Ocular Infections

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Abstract Herpes simplex virus type 1 (HSV-1) and its closely related herpes simplex virus type 2 (HSV-2) cause important clinical manifestations in humans including acute ocular disease and genital infections. These viruses establish latency in the trigeminal ganglionic and dorsal root neurons, respectively. Both viruses are widespread among humans and can frequently reactivate from latency causing disease. Currently, there are no vaccines available against herpes simplex viral infections. However, a number of promising vaccine approaches are being explored in preclinical investigations with few progressing to early-phase clinical trials. Consensus research findings suggest that robust humoral and cellular immune responses may partially control the frequency of reactivation episodes and reduce clinical symptoms. Live-attenuated viral vaccines have long been considered as a viable option for generating robust and protective immune responses against viral pathogens. Varicella zoster virus (VZV) belongs to the same alphaherpesvirus subfamily with herpes simplex viruses. A live-attenuated VZV vaccine has been extensively used in a prophylactic and therapeutic approach to combat primary and recurrent VZV infection, indicating that a similar vaccine approach may be feasible for HSVs. In this review, we summarize preclinical approaches to HSV vaccine development and current efforts to test certain vaccine approaches in human clinical trials. Also, we discuss the potential advantages of using a safe, live-attenuated HSV-1 vaccine strain to protect against both HSV-1 and HSV-2 infections.

Keywords Herpes simplex · Vaccine · Ocular · Genital · Keratitis · Live-attenuated · Immune evasion

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Introduction

The Alphaherpesviruses The *Alphaherpesvirinae* subfamily of *Herpesviridae* family contains the genera *Simplexvirus* (HSV) and *Varicellovirus* (varicella zoster virus (VZV)). Generally, alphaherpesviruses replicate rapidly in infected cells causing extensive cytolysis within 24 h post infection. An important property shared by all alphaherpesviruses is their ability to infect neurons establishing latency primarily, but not exclusively, in sensory ganglionic neurons. Herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) cause orofacial cold sores and severe ocular disease and blindness as well as genital ulcers, while VZV causes chickenpox/shingles in naïve younger individuals and in adult patients with weakened immune systems. HSVs are the prototypic viruses of the alphaherpesvirus subfamily, which also contains economically important animal viruses including Marek's disease-like virus (MDV), bovine herpesvirus type 1 (BHV-1), pseudorabies virus (PRV), and others. Alphaherpesviruses, as it is the case with many other viruses, have evolved specialized functions to subvert the host immune responses facilitating the establishment of latency in sensory neurons. Perturbations of the host immune system can often lead to viral reactivation from latency, suggesting the presence of an elaborate virally specified system for sensing host immune status particularly in the context of immune system interaction with neuronal cells.

HSV Infectivity HSV-1 enters neuronal cells via a pH-independent fusion of the viral envelope with neuronal plasma membranes, but it can enter a wide range of non-neuronal cells via either pH-independent or pH-dependent endocytosis [1–3]. Initial binding of gD to its cognate receptors including nectin-1, herpesvirus entry mediator (HVEM), and other receptors [4–9] is thought to trigger sequential conformational changes first in gD and then in gH/gL and ultimately gB that results in fusion of the viral envelope with cellular membranes during virus entry as well as fusion among cellular membranes [10–14]. Specifically, initial attachment of the virus to cellular membranes is mediated by interaction of glycoproteins gB and gC with glycosaminoglycan (GAG) moieties of cell surface proteoglycans [15, 16]. Subsequently, viral glycoprotein gD binds with one or more of its specific receptors, including the HVEM (HveA), nectin-1 (HveC), or 3-O-sulfated heparan sulfate [5–7]. gB can also bind to additional receptors (co-receptors), including paired immunoglobulin-like type 2 receptor alpha (PILRα), non-muscle myosin heavy chain IIA (NMHC-IIA), and myelin-associated glycoprotein (MAG), that play a pivotal role in virion attachment and virus entry [17–19].

Although gB is the sole fusogenic viral glycoprotein-mediating membrane fusion of the viral envelope with cellular membranes during virus entry as well as virus-induced cell-to-cell fusion that facilitates virus spread, viral glycoproteins gH, gL, and gK play accessory roles in controlling gB-mediated membrane fusion [13, 20]. Virions that lack gK enter into green African monkey kidney cells (Vero), albeit with lower efficiency than the wild-type virus [21, 22]. Deletion of amino acids 31–68 within the amino terminus of gK inhibits virus-induced cell-to-cell fusion and virus entry without drastically inhibiting virion envelopment and egress [20, 22]. Of particular interest is gK, which contains determinants that are required for successful infection of neuronal axons. Specifically, a recombinant virus lacking gK amino acids 31–68 replicated fairly efficiently in all cell types, while it was unable to establish latency after ocular infection of mouse eyes [23]. Recent experiments have shown that the gKΔ31–68 mutation prevents the virus from entering into axonal compartments of neurons in cell culture (manuscript, submitted).

After fusion of the viral envelope with the host plasma membrane, the tegumented capsids containing the viral genome are released into the cytosol and are transported via the microtubular network in a retrograde manner towards the nuclei of infected cells facilitated via the dynein-dynactin motor complex, which attaches tegumented capsids to microtubules [24–26]. The dynein-dynactin motor complex is utilized for the intracellular transport of other viruses including vaccinia virus and adenovirus [27–31].

HSV-1 and HSV-2 Pathogenesis HSV-1 and HSV-2 are closely related viruses with viral genomes exhibiting 83 % nucleotide identity [32]. However, these viruses cause different disease symptoms. HSV-1 causes cold sores, herpetic whitlow, encephalitis, herpes ocular infections, and keratitis [33–35], and it is the leading cause of infectious blindness in the USA [36, 37]. HSV-2 is primarily a sexually transmitted disease with high global prevalence, and the disease is primarily restricted to the genitals [38]. Seroprevalence studies have indicated that one of two adults in the USA aged 14–49 years old is infected with HSV-1 in a latent state [39]. HSV-1 has been increasingly identified as the cause of clinical genital herpes infections [40–42]. Infected individuals typically experience frequent but asymptomatic viral reactivation from latency, resulting in virus shedding that contributes to high transmission rates [43–45]. Both HSV-1 and HSV-2 produce persistent lifelong infections by establishing latency in immune-privileged sensory neurons [46]. Herpes infections can carry significant social implications, and the economic costs associated with genital herpes is substantial (projected to be around $2.5 billion in 2015 and around $3 billion in 2025) [47]. Importantly, genital HSV infection is considered a risk factor for acquiring human immunodeficiency virus infection [48–56] and, in some geographical areas, HSV-2 infection may be a contributing factor to 30–50 % of new HIV infections [49, 57]. A successful vaccination strategy against HSV-2 infection is predicted to have a dramatic global impact on HIV spread and prevention of genital clinical disease and neonatal infections [58–60].

Immune Responses Prior HSV infection appears to partially protect against re-infections and may decrease clinical disease symptoms including frequency of viral reactivation [43, 61]. Primary infection can induce strong humoral and cellular immune responses that contribute to controlling subsequent infections. The production of neutralizing antibodies is thought to play an important role in limiting virus spread [62–64]. However, adapted cellular immune responses exerted at mucosal sites of viral infection (ocular, genital) are crucial in controlling HSV infections as well as reactivation of the virus from latently infected ganglionic neurons [65–71].

Innate Immune Responses Virion particles are initially detected by pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) including viral antigens and viral DNA and RNA. As is the case with many infectious agents, innate immune responses mediated by macrophages, neutrophils, and other innate immune cells confer immediate nonspecific protection against HSV-2 [72]. Moreover, elicitation of the appropriate innate immune response may be required for the induction of downstream humoral and cellular immune responses. Glycoprotein gB binds Toll-like receptor 2 (TLR-2) and alters downstream signaling, which may be used for immune evasion purposes [73]. Other Toll-like receptors including TLR9 [74], TLR3 [75], and

TLR5 [76] appear to be involved in the immune response against HSVs.

Humoral Immune Responses Although preclinical studies have indicated that strong humoral responses may limit viral spread and disease symptoms, experimental infections in animals have also shown that B cells were dispensable with relation to clearance of HSV-2 infection [77] and human clinical trials of certain vaccines have failed despite the generation of strong humoral responses against the virus [78, 79]. Other experiments have shown that humoral responses may contribute to disease protection [62, 80–83]. A replication-defective HSV-2 lacking gD2 was able to protect mice against lethal genital HSV-2 challenge. This vaccine produced very weak neutralizing antibodies against the challenging virus; however, serum from vaccinated animals was able to passively protect naïve mice from challenge. The serum displayed potent antibody-dependent cellular cytotoxicity properties targeting antigens other than gD2 [84]. Collectively, it is highly probable that humoral responses play a significant role in limiting HSV viral infections and associated clinical symptoms.

Cellular Immune Responses Extensive work in experimental animals has provided strong evidence that induction of both HSV-specific $CD4^+$ T cells and $CD8^+$ T cells are required for maximum protection [69, 77]. Data from subsequent studies indicate that $CD8^+$ tissue-resident T cells may protect against HSV-2-reactivated virus in human peripheral tissues [85]. The importance of tissue-specific memory immune responses is underscored in a recent study that has utilized a "prime-pull" immunogenicity approach consisting of initial parenteral vaccination to induce systemic T cell response followed by intravaginal chemokine application to enhance recruitment of activated T cells to genital tissues [86]. Several studies have also implicated the involvement of regulatory T cells (Tregs) in the control of HSV-2 infections, since in Treg-deprived mice, dendritic and T cells appeared to migrate more slowly into infected tissues and interferon levels were lower in the draining lymph nodes [74].

Vaccine Approaches Many vaccine approaches and candidate vaccines have been tested in laboratory animals and humans including purified peptides; recombinant glycoprotein subunits; inactivated, live-attenuated, replication-competent and replication-defective whole virus; as well as DNA-based vaccines administered via different routes of immunization (reviewed in [59, 87–92]). A detailed chronological list of published papers during the last 5 years is provided in Table 1, and certain distinct vaccine approaches are described below.

Subunit and Peptide Vaccines Subunit vaccines are the most studied HSV-2 vaccines; however, to date, none have conferred protective immune responses against HSV-2 infections in clinical trials [135]. Viral glycoproteins gB and gD that can elicit both humoral and cellular immune responses have been favored as subunit vaccines. A gD2 subunit vaccine with an alum/MPL adjuvant reduced HSV-2 disease in HSV-2-seronegative women but had no apparent benefit against genital herpes in men and HSV-1-seropositive women [136]. A subsequent double-blind, controlled, randomized efficacy field trial of a HSV-2 glycoprotein D (gD-2) subunit vaccine (Herpevac Trial) in 8323 women showed that the vaccine was 82 % protective against HSV-1 genital disease but offered no significant protection against HSV-2 genital disease [79]. Antiviral protection correlated with induction of neutralizing antibody against gD-2, while cellular immune responses did not appear to play an important role in conferring protection [137, 138].

Newer vaccine approaches utilize libraries of B and T cell epitope peptides in conjunction with newer adjuvant formulations that are specifically targeted to elicit strong humoral and cellular immune responses. An example of this approach is the use of T cell epitopes derived from the ICP4 protein and antibody generated by the gD2 glycoprotein in conjunction with the proprietary adjuvant Matrix-M (reviewed in [88]) (Table 2, GEN-003). Recently, it was recognized that T cells from symptomatic and asymptomatic patients target different epitopes, leading to the hypothesis that elicitation of the appropriate B and T cell responses may provide protection [139]. A vaccine approach based on this principle (HerpV) generated $CD4^+$ and $CD8^+$ T cell responses in mice and in HSV-2-seropositive human patients [140, 141] (Table 2).

DNA and Viral-Vectored Vaccines DNA vaccine vectors expressing gD2 and gB2 genes have shown an ability to elicit protective immune responses in preclinical studies. A DNA-based gD2 vaccine has been shown to elicit cellular immune responses in a double-blind, vehicle-controlled, dose escalation safety, and immunogenicity trial [142]. However, it is unlikely that this type of vaccine approach will find wide use, since typically multiple vaccinations are needed over a prolonged period of time to elicit significant immune responses. HSV viral antigens also have been expressed via a variety of viral vectors including adenoviruses and vaccinia virus that can induce strong adjuvant-like responses in experimental animals and humans. Specifically, the modified vaccinia virus Ankara (MVA) has been used to express HSV-2 gD, inducing strong cellular and humoral immunity [143].

Live-Attenuated Virus Vaccines In principle, live-attenuated vaccines have distinct advantages over subunit and inactivated vaccines, primarily because replication of the pathogen allows for the entire repertoire of pathogen-specific antigen expression. Given the 83 % nucleotide identity shared by both HSV-1 and HSV-2 genomes [32], cross-protective immunity may

Table 1 Past 5 years of preclinical HSV vaccine development

Type	Description	Adjuvant	Animal model	Route of challenge	Year	Reference
Epitope	$gD2_{53–61}$, $gD2_{70–78}$, $gD2_{278–286}$, $gD2_{287–317}$	*N*-Palmitoyl-lysine	Rabbit	Ocular	2015	[93•]
Epitope	HSV-1 $VP11/12_{66–74}$, $VP11/12_{220–228}$, $VP11/12_{702–710}$	CpG	Mouse	Ocular	2015	[94]
Replication defective	HSV-2 DeltagD(−/+gD1)	NA	Mouse	Genital	2015	[84]
Replication defective	HSV-2 HSV529	NA	Guinea pig/mouse	Genital	2015	[95]
Live attenuated	HSV-2 ICP0(−) virus, 0DeltaNLS	NA	Mouse	Genital	2015	[96]
Recombinant virus	HPV-gBsec, HPV-gDsec, HPV-gBsec/gDsec	NA	Mouse	Genital	2015	[97]
Live attenuated	HSV-1 KOS-63	NA	Mouse	Ocular	2014	[98]
Live attenuated	HSV-1 HSV-CD80	NA	Mouse	NA	2014	[99]
Subunit	gG2	CpG/alum	Mouse	Genital	2014	[100]
Subunit	gD2, gD2/gC2	CpG/alum, MPL/alum	Guinea pig	Genital	2014	[101]
Subunit	gD2, gE2/gC2/gD2	CpG/alum	Mouse	Genital	2014	[102]
Replication defective	HSV-2 CJ2-gD2	NA	Guinea pig	Genital	2014	[103]
Live attenuated	HSV-2 UL24 mutant	NA	Guinea pig/mouse	Genital	2014	[104]
Live attenuated	HSV-1 VC2	NA	Mouse	Genital	2014	[105••]
Live attenuated	HSV-1 encoding the HIV-1 Tat	NA	Mouse	Genital	2014	[106]
Live attenuated	HSV-2 TK(−)	NA	Mouse	Genital	2014	[107]
Epitope	$gB1_{342–350}$, $gB1_{561–569}$, $gB1_{183–191}$, $gB1_{441–449}$	CpG	Mouse	Ocular	2013	[108]
DNA	pIRES I and pIRES II	NA	Mouse	Nasal	2013	[109]
Other	DC/HSV-1	NA	Mouse	IP, ear	2013	[110]
Replication defective	HSV-1 Δ gK	NA	Mouse	Genital	2013	[111]
Live attenuated	Wild-type HSV-2, HSV-2 ICP0 (−) viruses (0Delta254, 0Delta810, 0DeltaRING, or 0DeltaNLS)	NA	Guinea pig	Genital	2013	[81]
DNA	Ubiquitinated and non-ubiquitinated constructs encoding gD	NA	Mouse	Genital	2013	[112]
Other	DTK-NISV, gB1s-NISV	NA	Mouse	Genital	2013	[113]
Live attenuated	HSV2-gD27	NA	Mouse	NA	2012	[114]
Subunit	gD2	IC31((R))	Mouse	Genital	2012	[115]
Replication defective	HSV-2 ACAM529	NA	Mouse	Genital	2012	[116]
Live attenuated	HSV-2 gE2-del	NA	Guinea pig/mouse	Genital	2012	[117]
Recombinant virus	Lipo/rAdv5	NA	Mouse	Genital	2012	[118]
Replication defective	HSV-1 ICP8(−)vhs(−) and ICP8(−)vhs(−)B7	NA	Mouse	Ocular	2011	[119]
DNA	pRSC-gD-IL-21	NA	Mouse	Ocular	2011	[120]
DNA	pRSC-gD-IL-21	NA	Mouse	Ocular	2011	[121]
DNA	pcgB, pcEpitope	NA	Mouse	NA	2011	[122]
Subunit	gD2, truncated gD2t, gD2/gB2/gH2/gL2, gB2/gH2/gL2	CLDC	Guinea pig	Genital	2011	[123]
Subunit	gD2, gB2, gD2/gB2	CpG/alum	Guinea pig/mouse	Genital	2011	[124]
Subunit	gC2/gD2	CpG/alum	Guinea pig/mouse	Genital	2011	[125]
Epitope	$gB2_{466–473}$, $gC2_{216–223}$, $gD2_{6–18}$, $gE2_{483–491}$, $gG2_{572–579}$, $gI2_{286–295}$, $gD2_{21–28}$, $gB2_{162–177}$, $gD2_{205–224}$, $gD2_{245–259}$, $gD2_{10–20}$, $gD2_{268–276}$	Freund's adjuvant	Mouse	Genital	2011	[126]
Live attenuated	HSV-2 ICP0(−) virus, 0DeltaNLS	NA	Mouse	Genital	2011	[127]
DNA	gD DNA vaccine	NA	Mouse	Genital	2011	[128]
DNA	pVAX-FI-HSV2	NA	Guinea pig	Genital	2011	[129]
Other	pcDNA3-gD phagemid particles	NA	Mouse	NA	2010	[130]
Subunit	gD2	CLDC, MPL/alum	Guinea pig	Genital	2010	[131]
Replication defective	HSV-2 dl5-29, dl5-29-41, dl5-29-41.1	NA	Mouse	Genital	2010	[132]
Live attenuated		NA	Mouse	Genital	2010	[133]

Table 1 (continued)

Type	Description	Adjuvant	Animal model	Route of challenge	Year	Reference
	wild-type HSV-2, HSV-2 ICP0 (−) viruses (0Delta254, 0Delta810, 0DeltaRING, or 0DeltaNLS)					
DNA	Nanopatch+DNA vaccine	NA	Mouse	Genital	2010	[134]

Table 2 Current clinical trials of HSV vaccines

Intervention	Company	Trial	Info	ClinicalTrials.gov identifier
Replication-defective HSV529	Sanofi Pasteur	Ongoing phase 1	The trial, being conducted at the National Institutes of Health Clinical Center, is utilizing healthy adults aged 18–40 years and consist of 3 vaccination visits with 7 follow-up visits. Participants will be screened with a medical history and physical exam. The study has an estimated completion date of January 2030	NCT01915212
HerpV polyvalent peptide complex adjuvanted with QS-21	Agenus	Completed phase 2	Enrolled a total of 80 subjects aged 18 to 50 years with a history of 1–9 herpes episodes within the prior 12-month period. Treatment consisted of 3 injections of HerpV at a dose of 240 μg in 2-week intervals. Results published in a press release showed that vaccination with HerpV demonstrated a significant reduction in viral shedding (P=0.015; RR=0.85). These results suggest a 15 % reduction in viral shedding and a 34 % reduction in viral load (P=0.08)	NCT01687595
GEN-003 is a subunit vaccine comprised of HSV-2 glycoprotein D2 ($gD2\Delta TMR_{340–363}$) and infected cell polypeptide 4 ($ICP4_{383–766}$) adjuvanted with proprietary Matrix-M2	Genocea	Ongoing phase 2	Press release of results from the phase 1/2a clinical trial demonstrated that during a 28-day observation period 6 months after administration of GEN-003 patients that received 30 μg doses presented a 65 % reduction in genital lesion occurrence, significantly less than baseline (P<0.001). Observations collected again 12 months after vaccination demonstrated a 42 % reduction on lesion formation and significantly elevated humoral and cellular immune responses. The phase 2 study consists of 310 subjects from 17 institutions in the USA. Topline results which identified an optimal dose of 60 μg per protein/75 μg of Matrix-M2 adjuvant demonstrated a highly statistically significant (P<0.0001) 55 % reduction from baseline in the viral shedding rate, the primary endpoint of the trial and a measure of anti-viral activity	NCT02114060
VCL-HB01 plasmid-based vaccine encoding two HSV-2 proteins and VCL-HM01 plasmid-based vaccine encoding one HSV-2 protein, both adjuvanted with Vaxfectin	Vical	Ongoing phase 1/phase 2	A press release has stated that Vical has completed enrollment in its clinical study, and the randomized, double-blind, placebo-controlled trial will evaluate safety, tolerability, and efficacy of two vaccine candidates (one encoding glycoprotein D alone and the other in combination with UL46). The study is powered to show at least a 30 % decrease in the viral shedding rate following 3 doses of vaccine. A total of 165 otherwise healthy HSV-2-infected patients aged 18 to 50 years were enrolled across seven US trial sites. The company expects to release efficacy data in the middle of 2015	NCT02030301

be achieved by a single safe and efficacious vaccine expressing a large enough repertoire of cross-protective antigens. Attempts at generating a live-attenuated HSV vaccine have focused on the preparation of attenuated viruses that can generate robust immune responses while minimizing potential virulence in the host. Generally, the entire genes that play important roles in the virus life cycle have been deleted or otherwise modified to attenuate the virus and allow a more robust production of humoral and cellular immune responses. Viral genome modifications include deletions in glycoprotein E (gE) [117, 144]; multiple deletions in γ34.5, UL55-56, UL43.5, and US10-12 genes [145] and in UL5, UL29, UL41, and ICP27 genes [146–149]; deletion of ICP0 [127, 133] and UL9 genes [150–153]; and deletion of gD [84]. Other live virus vaccines under study include the HSV-1 virus CJ9-gD engineered to overexpress gD1 and having a dominant negative mutation to prevent virus replication. This vaccine strain has been reported to protect guinea pigs from HSV-2 intravaginal challenge, with marked reduction in vital titer and lesion formation [152]. These vaccine approaches face two major hurdles that need to be overcome before they can be successfully applied to human patients: (1) replication-defective viruses typically require growing the virus in a complementing cell line adding to the complexity of producing the vaccine as well as associated safety and regulatory issues associated with the validation of the cell line that is used to grow the virus and (2) all viruses, regardless of the specific gene deletions, are capable of entering into neurons and may establish latency as well as potentially recombine with endogenous HSVs. To circumvent this potential safety issue, a recombinant virus carrying specific mutations in gD2 that prevent the virus for binding to its neuronal receptor nectin-1 was recently constructed and shown to protect mice against HSV-2 infection, while the vaccine virus did not enter into ganglionic neurons [114]. However, these single-amino acid changes in gD can revert in vivo to produce a wild-type-like virus with pathogenic potential.

Initial experiments with live-attenuated vaccination of human patients with a HSV-2 mutant virus, which is deleted in the PK domain of the large subunit of ribonucleotide reductase (ICP10DeltaPK) [154], revealed that only a fraction of the vaccinated individuals were significantly protected against viral reactivation and subsequent clinical symptoms [155]. Similarly, a virus having the gH gene deleted revealed no significant benefit against HSV-2 infection in immunocompetent men and women [156]. A current clinical trials involves the testing of replication-defective HSV529, a vaccine version derived from mutant virus dl5-29 virus having the UL29 (ICP8) and UL5 (component of the viral helicase-primase), which has been shown to induce protective immune responses in mice and guinea pigs [95, 132]. However, deletion of the UL29 and UL5 genes will not prevent virus entry into neurons and establishment of latency. Current clinical trials with various vaccine approaches are listed in Table 2.

Generation of a safe and effective replication-competent HSV-1 virus is important to not only vaccinate against acquiring HSV infection and reduce HIV prevalence, but also a safe vaccine vector could be utilized for expression of heterologous antigens from other pathogens. HSV has many non-essential genes and can stably carry large fragments of foreign DNA. This genetic flexibility is ideal for the expression of antigens specific to other pathogens [157, 158]. The recombinant HSV-1 talimogene laherparepvec (Amgen, Inc.) expressing granulocyte monocyte colony-stimulating factor (GM-CSF), a potent chemokine functioning in the maturation of macrophages, has been used in combination with other chemotherapeutics for the treatment of squamous cell cancer of the head and neck with promising phase I/II results [159]. In addition, this vaccine improved durable response rate in patients with advanced melanoma in a phase III clinical trial [160•]. FDA approval of talimogene laherparepvec for melanoma therapy is expected to pave the way for the use of live-attenuated HSV-based vectors for vaccination against HSV and other pathogens.

Lessons Learned from the Live-Attenuated VZV Vaccine Strain The VZV alphaherpesvirus causes varicella (chickenpox) during primary infections followed by establishment of latency in dorsal ganglionic neurons. Reactivation of the virus causes herpes zoster (HZ), commonly referred to as shingles. The currently used Oka vaccine strain of VZV was isolated from a healthy Japanese child with varicella and attenuated by serial passage in cell culture. The vaccine was initially tested for a number of years in Japan and later gained FDA approval for use in Europe and the USA. The vaccine has been proven to be safe and provide significant clinical efficacy in immunocompetent individuals, while it was shown to boost VZV-specific cell-mediated immune responses in both immunocompetent and immunocompromised adults. Routine childhood immunization has markedly reduced the incidence of varicella in the USA [161–164].

Development of the HSV-1 VC2 Vaccine Strain Previously, we have shown that a HSV-1 gK-null virus was unable to infect ganglionic neurons and establish latency after ocular infection of mice [165, 166]. Recently, we capitalized on the attenuated properties of the gK-null virus and showed that intramuscular vaccination of mice with the gK-null virus conferred significant cellular immune responses and protection against intravaginal challenge of mice with either virulent HSV-1(McKrae) or HSV-2(G) viruses [111]. To further improve this vaccination approach, we constructed the VC2 mutant virus with specific deletions within the genes coding for glycoprotein K (gK) and UL20. The VC2 virus contains the gKΔ31–68 mutation that prevents the virus from infecting

ganglionic neurons after ocular infection in mice [167]. In contrast to the gK-null virus that requires replication in the complementing cell line VK302 that expresses gK, the VC2 virus can replicate efficiently in infected Vero cells, achieving titers similar to those of the wild-type HSV-1(F) parental virus in cell culture [105••, 111]. A single intramuscular vaccination with the VC2 virus was very well tolerated at a high infectious dose (10^7 PFU), produced a robust humoral and cell-mediated immune response, and conferred 100 % protection against lethal intravaginal challenge with either HSV-1 (McKrae) or HSV-2 (G) viruses [105••].

Amelioration of herpetic eye disease can be augmented either prophylactically or therapeutically by a robust anti-HSV immune response [93•, 94, 108, 119–121] (Table 1). Specifically, an asymptomatic individual's immune system recognizes a different repertoire of HSV antigens than those of a symptomatic individual [168–170]. Alteration of these antigenic recognition patterns within target HSV proteins must be induced during the primary insult of infection. VC2 exhibits distinctly different innate recognitions when compared to the parental virus HSV-1(F) strain and can protect against lethal ocular infection of mice, suggesting that gK is involved in immune evasion (unpublished). This altered innate recognition of the VC2 virus may serve as a stimulus for significantly altered epitope recognition in the downstream adaptive immune response, resulting in enhanced immune responses against HSV-1 and HSV-2 ocular and genital infections.

Conclusions

Based on the successful deployment of the VZV live-attenuated vaccine, it is likely that a similar approach could be used to produce an efficacious live-attenuated HSV vaccine provided that safety concerns are resolved. Preclinical results with the VC2 vaccine are particularly encouraging. The VC2 vaccine, unlike other live-attenuated viral strains, cannot enter into neuronal axons and elicits stronger humoral and cellular immune responses than its parental HSV-1(F) strain, indicating that the specific modifications of the VC2 gK and UL20 proteins alter the canonical signaling pathways that ultimately produce robust humoral and cellular immune responses. Understanding immune evasion mechanisms mediated by gK and UL20 in conjunction with other viral glycoproteins may provide improved versions of the VC2 vaccine strain that can elicit strong, tissue-specific protective B and T cell responses. Also, VC2 could serve as a vector platform for the production of vaccines against other viral and bacterial infections.

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Compliance with Ethics Guidelines

Conflict of Interest Dr. Kousoulas and B. Stanfield report that a patent application on the VC2 vaccine is pending.

Human and Animal Rights and Informed Consent This article contains no studies with human or animal subjects performed by the author.

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- • Of importance
- •• Of major importance

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