Combinatorial Herpes Simplex Vaccine Strategies: From Bedside to Bench and Back

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The development of vaccines against herpes simplex virus type 1 and type 2 (HSV1 and HSV-2) is an important goal for global health. In this review we reexamined (i) the status of ocular herpes vaccines in clinical trials; and (ii) discusses the recent scientific advances in the understanding of differential immune response between HSV infected asymptomatic and symptomatic individuals that form the basis for the new combinatorial vaccine strategies targeting HSV; and (iii) shed light on our novel “asymptomatic” herpes approach based on protective immune mechanisms in seropositive asymptomatic individuals who are “naturally” protected from recurrent herpetic diseases. We previously reported that phenotypically and functionally distinct HSV-specific memory CD8⁺ T cell subsets in asymptomatic and symptomatic HSV-infected individuals. Moreover, a better protection induced following a prime/pull vaccine approach that consists of first priming anti-viral effector memory T cells systemically and then pulling them to the sites of virus reactivation (e.g., sensory ganglia) and replication (e.g., eyes and vaginal mucosa), following mucosal administration of vectors expressing T cell-attracting chemokines. In addition, we reported that a combination of prime/pull vaccine approach with approaches to reverse T cell exhaustion led to even better protection against herpes infection and disease. Blocking PD-1, LAG-3, TIGIT and/or TIM-3 immune checkpoint pathways helped in restoring the function of antiviral HSV-specific CD8⁺ T cells in latently infected ganglia and increased efficacy and longevity of the prime/pull herpes vaccine. We discussed that a prime/pull vaccine strategy that use of asymptomatic epitopes, combined with immune checkpoint blockade would prove to be a successful herpes vaccine approach.

Keywords: herpes simplex virus, clinical trials, vaccines, asymptomatic, immune checkpoint blockade
INTRODUCTION

According to the World Health Organization (WHO), over two-thirds of the worldwide population in infected with HSV-1 (commonly known to cause oral herpes or cold sores) and HSV-2 (commonly known to cause genital herpes) (1, 2). The prevalence of HSV-1 and HSV-2 is 47.8% and 11.9%, respectively, for individuals aged 14 to 49 years according to a 2018 February data brief published by the US Centers for Disease Control and Prevention’s National Center for Health Statistics (1, 2). In the United States alone, every year, there are 500,000 HSV-1 oral herpes cases; 300,000 HSV-1 and HSV-2 genital herpes cases; 20,000 HSV-1 ocular herpes cases and 1,500 cases of herpes encephalitis (3, 4). Apart from being the most prevalent sexually transmitted disease, HSV-1 is the leading cause of infectious blindness in Western countries (5). HSV-1 and HSV-2 are neurotropic viruses that infect the anogenital, oral mucosal lining and the skin and the eyes (6). The immune response to HSV typically controls the acute mucosal infection; however, the virus remains latent in the ganglia, and there is a life-long sporadic low-grade shedding of virus from sensory neurons into the mucosa (6). Thus, while HSV hides for a lifetime in the trigeminal, autonomic, or dorsal root ganglia, it reactivates and sheds asymptomatically making the transmission high. In addition to causing painful blisters, HSV-2 can cause encephalitis and death in newborns from vertical transmission and increases the risk for HIV infection two-three-fold times (7).

Antiviral drugs are the only current treatment approved by the Food and Drug Administration (FDA) for treatment of herpetic diseases. Due to the cost, virus resistances and limited effectiveness of antiviral drugs, preventive or therapeutic vaccines are highly desirable to control herpes infection and/or diseases (8). The development of a vaccine that proves effective against one type of the HSV would be helpful for the other type due to the genetic similarity between HSV-1 and HSV-2. However, due to virus latency and HSV immune evasion, immunotherapy and vaccine development against the virus have become a real challenge. As of 2018, a number of different HSV vaccine candidates were at different stages of clinical trials (9, 10) (Table 1).

One common denominator in these vaccines is the use of the whole virus or whole virus proteins, which contain both protective “asymptomatic” epitopes and pathogenic “symptomatic” epitopes. Our developed “asymptomatic” herpes vaccine approach which is based on understanding the immune mechanisms by which seropositive asymptomatic individuals are “naturally” protected from recurrent herpes disease throughout their lives. Clinical and pre-clinical studies have proved that the T cell-based immune system in the mucosa lining of the genital tract plays a crucial role in the prevention of HSV acquisition. A better mucosal vaccine approach to boost effector memory T cell responses will serve instrumental in developing an effective HSV vaccine (45). Our latest approach of using adenoviral vectors delivering chemokines and asymptomatic dominant epitopes to induce and pull antiviral CD4+ and CD8+ T cells to the site of reactivation (i.e., ganglia) and replication (i.e., epithelia) would be an effective combinatorial herpes simplex vaccine strategy. Moreover, another combinatorial herpes simplex vaccine strategy that consists of reversing T cell exhaustion by immune checkpoint blockade would be a successful strategy to clear herpes infection (46). In this review, we highlight the current clinical trials in herpes vaccine development and emphasize the significance of using the asymptomatic epitope approach in a combinatorial vaccine strategy.

HSV VACCINES: FROM PAST TO PRESENT

The success of vaccines against other alpha herpes, like the chicken-pox and shingles vaccine, has given hope for the development of a vaccine against HSV (47) (Table 1). Four main vaccine approaches have been designed and tested in the past four decades to fight off herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) infections and diseases (48): (1) Inactivated “killed” HSV vaccines; (2) Live-attenuated HSV vaccines; (3) Replication-defective HSV vaccines; and (4) Subunit HSV vaccines (9, 49–54). Each of these types of vaccine approaches has its pros and cons when it comes to safety, immunogenicity, and protective efficacy.

Inactivated “Killed” HSV Vaccines

HSV is a highly successful neurotropic virus that resides in the nervous system and therefore presents the risk of developing neuro-pathogenesis and life-threatening Herpes Simplex Encephalitis (HSE). Thus, back in the 70s and 80s, the first whole inactivated HSV vaccine approach used “kill” the whole virus after exposure to heat, UV-light (55) or chemicals (56, 57). These whole inactivated HSV vaccines induced antibodies, but not T cells, and as such have not been successful in the protection against recurrent HSV-1 or HSV-2 infections and diseases (58–60). Therefore, the live-attenuated HSV vaccines (61–66) and replication-defective HSV vaccines were introduced (51, 58–60, 67–71).

Live-Attenuated HSV Vaccines

Live-attenuated HSV vaccines contrast inactivated HSV vaccines produced by “killing” the virus and reducing the neurovirulence of HSV-1 or HSV-2, while keeping them viable. In the past 24 years, many live-attenuated HSV vaccines have been introduced and tested in both the mouse and guinea pig models mainly in a prophylactic setting (instead of a therapeutic setting). However, due mostly to safety concerns, only a few of these live vaccines have progressed into clinical trials (63). Live-attenuated HSV vaccines include: (1) The HSV-2 TK- mutant reported back in 1995 by Milligan and Bernstein and then by Kiyono in 2014 (72); (2) the RAV 9395 live attenuated recombinant virus; evaluated in guinea pigs and reported by Spaete back in 1998 (70); (3) AD472, a live attenuated recombinant HSV-2 vaccine evaluated in guinea pigs was reported back in 2005 (51); (4) The most studied HSV-1 and HSV-2 ICP0 (-) live-attenuated mutant vaccines, lacking the
| Type of Vaccine                  | Vaccine Construct                    | Administration Route | Phase of Trial | Virus Subtype | Results                                                                 | Limitations                                                                                   | Ref. |
|---------------------------------|--------------------------------------|----------------------|----------------|---------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|------|
| Inactivated Vaccine HSV-1 gH deletion (SC164gH) | Subcutaneous in human                 | Clinical trial       | HSV-2           | Unable to show protection against acute or recurrent genital herpes infection | Vaccine did not achieve clinical usefulness; Alternative approaches could be proposed           | (11) Akhranueva NV, Zhang P, Sugiyama N, Behar SM, Yao F. Development of a glycoprotein D- expressing dominant-negative and replication-defective herpes simplex virus 2 (HSV-2) recombinant viral vaccine against HSV-2 infection in mice. J Virol, 85(10), 5036-5047 (2011). |
|                                | Subcutaneous and intravaginal in guinea pig | Preclinical trial    | HSV-2           | Provides complete protection against primary and recurrent HSV infection | Missing reproducibility on correlation between antibody titers and recurrent infection pattern       | (12) Reszka NJ, Dudek T, Nilese DM. Construction, and properties of a herpes simplex virus 2 d55-29 vaccine candidate strain encoding an HSV-1 virion host shutoff protein, Vaccine, 28(15), 2754-2762 (2010). |
|                                | Intraepithelial and intravaginal in guinea pig | Preclinical trial    | HSV-2           | Provides high potency for complete HSV protection | The immune mechanisms involved in the control of recurrent infection need to be elucidated      | (13) Belshe PB, Leone PA, Bernstein DI et al. Clinical trial HSV-2 multiple genes deletion of HSV-2 | 
|                                | Scanification via ear pinna route in mice | Preclinical trial    | HSV-1           | Establishes self-limiting HSV infection | High risk of genetic recombination; Unable to block the virus reactivation to prevent disease recurrences; This study needs more animal experiment for statistical significance | (14) Bernard MC, Barbian V, Pradzynski F et al. Immunogenicity, protective efficacy, and non-replicative status of the HSV-2 vaccine candidate HSV529 in mice and guinea pigs. PLoS One, 10(4), e0121518 (2015). |
|                                | Subcutaneous in mice                  | Preclinical trial    | HSV-2           | Reduces HSV symptoms; Lowers HSV related genital and neurological disease | May reactivate latent HSV; May not affect viral shedding. The protective immunity mediated by antibody and T-cells | (15, 16) Ohashi M, Berti AS, Patel A, Krause PR. Spread of herpes simplex virus to the spinal cord is independent of spread to dorsal root ganglia. J Virol, 85(6), 3030-3032 (2011). Dasgupta G, Chentou AA, Kalantar M et al. Immunodominant “asymptomatic” herpes simplex virus 1 and 2 protein antigens identified by probing whole-ORFome microarrays with serum antibodies from seropositive asymptomatic versus symptomatic individuals. J VIROL, 86(8), 4358-4369 (2012). |
|                                | Multiple genes Deletion of HSV-2      | Subcutaneous in mice | Preclinical trial | Reduces viral titer and viral shedding | The genetic basis underlying the latency defect should be elucidated | (17) Dasgupta G, Nesburt AB, Wechsler SL, BenMohamed L. Developing an asymptomatic mucosal herpes vaccine: the present and the future. Future Microbiol, 5(1), 1-4 (2010). |
|                                | HSV-2 ICP10ΔPK deletion                | Subcutaneous in mice | Preclinical trial | Increases IL-12 secretion by DCs | Does not readily begin latency; Must show the frequency and duration of memory T-cells; Assess the ability to activate p38MAPK in T-cells | (18) Chentou AA, BenMohamed L. Future viral vectors for the delivery of asymptomatic herpes epidermal-based immunotherapeutic vaccines. Future virology, 5(5), 525-528 (2010). |
|                                | HSV-2 UL5 & UL29 genes deletion       | Intramuscular in humans | Clinical trial | Safe and well tolerated | More reactions than placebo on the injection site | (19) Schiffer JT, Abu-Raddad L, Mark KE et al. Mucosal host immune response predicts the severity and duration of herpes simplex | 

(Continued)
| Type of Vaccine | Vaccine Construct | Administration Route | Phase of Trial | Virus Subtype | Results | Limitations | Ref. |
|-----------------|-------------------|----------------------|----------------|---------------|---------|-------------|------|
| RAV9395 (Deletion of HSV-2 r134.5 gene, UL55 and UL56 ORF) | Intramuscular | Preclinical trial | HSV-2 | Decreases lesion development and HSV infection severity | Decreases frequency of HSV reactivation from explanted DRG | N/A | (23) Pope C, Kim SK, Marzo A et al. Organ-specific regulation of the C6E T cell response to Listeria monocytogenes infection. Journal of immunology, 169(3), 3402-3409 (2002). |
| VC2 (mutations in gK and UL20) | Intramuscular | Preclinical trial | HSV-1 and HSV-2 | Fully protects against lethal intravaginal HSV challenge | Presents cross-protective humoral and cellular immunity | N/A | (24) Gebhardt T, Whitney PG, Zaid A et al. Different patterns of peripheral migration by memory CD4+ and CD8+ T cells. Nature, 477 (7363), 216-219 (2011). |
| HSV-2 gE deletion | Footpad injection | Preclinical trial | HSV-2 | Decreases acute viral replication in vagina, amount of virus in neural tissue, subsequent recurrent disease, and viral shedding | Delivers protection after 6 months | N/A | (25) Nelson MH, Bird MD, Chu CF et al. Rapid clearance of herpes simplex virus type 2 by CD8+ T cells requires high level expression of effector T cell functions. J Reprod Immunol, 89(1), 10-17 (2011). |
| HSV-2 ICPO-ΔNLS | Intramuscular, intravaginal, and intravenous | Preclinical trial | HSV-2 | No disease mortality | Absence of infectious virus in DRG and recurrent HSV shedding in vagina | Provides incomplete protection | (Continued) |

(Continued)
### TABLE 1 | Continued

| Type of Vaccine | Vaccine Construct | Administration Route | Phase of Trial | Virus Subtype | Results | Limitations | Ref. |
|----------------|------------------|----------------------|----------------|--------------|---------|-------------|------|
| VC2 (gkD31-68 deletion of HSV-1) | Intramuscular | Preclinical trial | HSV-2 | - Shows poor HSV replication at the immunization site | - Rarely infects neural tissue | - Lack of any genital disease | - Reduces severity of acute and recurrent HSV-2 shedding in vagina and quantity of virus in DRG | - Better selection as a prophylactic vaccine | N/A | (30) Rott LS, Birsin MJ, Andrew DP, Berg EL, Butcher EC. A fundamental subdivision of circulating lymphocytes defined by adhesion to mucosal addressin cell adhesion molecule 1. Comparison with vascular cell adhesion molecule-1 and correlation with beta 7 integrins and memory differentiation. J Immunol, 156(10), 3727-3728 (1996). |
| R2 (HSV-1 mutation in region 2 of pUL37) | Intramuscular, intradermal, and intravaginal | Preclinical trial | HSV-2 | - Increases neutralizing antibody titers along with CD3+, CD4+ and CD8+ T-cells | - Decreases infiltration of Iba1+ macrophages | N/A | - T-cell response is only observed at a single time point | (31) Mebius RE, Streeter PR, Michie S, Butcher EC, Weissemann IL. A developmental switch in lymphocyte homing receptor and endothelial vascular addressin expression regulates lymphocyte homing and permits CD4+ CD8- cells to colonize lymph nodes. Proc Natl Acad Sci U S A, 93(20), 11019-11024 (1996). |
| HSV-1 ICPOΔNLS | Subcutaneous and intramuscular | Preclinical trial | HSV-1 | - Shows less infectious virus during acute infection in TG and brainstem | - Stimulates an immune response by increasing the gB-elicited interferon (IFN)-γ, granocyte B and CD107a; and decreasing LAG-3, PD-1, and TIM-3 | N/A | - Decreases in infiltration of Iba1+ macrophages | (33) Abitorabi MA, Mackay CR, Jerome EH, Osorio O, Butcher EC, Erle DJ. Differential migration properties of three major subsets of tissue homing T cells in sheep. Eur J Immunol, 26(10), 2433-2439 (1996). |
| Naked DNA vaccine | pSVL- HSV-1 gD, pRc/CMV- HSV-1 gD | Intramuscular | Preclinical trial | HSV-1 | - Provides low protection against HSV-1 | - Reduces acute and recurrent HSV-2 shedding, IgG and neutralizing antibody responses | (32) Mackay CR, Andrew DP, Birsin M, Ringer DJ, Butcher EC. Phenotype and migration properties of three major subsets of tissue homing T cells in sheep. Eur J Immunol, 26(10), 2433-2439 (1996). |
| | pDNA encoding HSV-2 gD2 | Intramuscular | Clinical trial | HSV-1+HSV-2-, HSV-2+HSV-2- | - Produces strong HSV-2-virus-specific IgG and neutralizing antibody responses | (34) von Andrian UH, Mackay CR, Jerome EH, Osorio O, Butcher EC, Erle DJ. Differential expression of homing molecules on recirculating lymphocytes from sheep gut, peripheral, and lung lymph. J Immunol, 150(9), 3111-3117 (1996). |
| | pDNAs encoding HSV-2 gD2 | Subcutaneous | Preclinical trial | HSV-2 | - Should be studied in a greater number of guinea pigs | (33) von Andrian UH, Mackay CR, T-cell function and migration. Two sides of the same coin. N Engl J Med, 343(14), 1020-1034 (2000). |
| | pDNA encoding HSV-2 gD2 coupled with Vaxfectin ® | Intramuscular | Preclinical trial | HSV-2 | - Increases IgG antibody titers | (35) Mackay UK, Waki K, van Vliet CJ et al. Maintenance of T cell function in the face of chronic antigen stimulation and repeated | |
| Vaccine Construct | Administration Route | Phase of Trial | Virus Subtype | Results | Limitations | Ref. |
|-------------------|----------------------|----------------|---------------|---------|-------------|-----|
| pDNA encoding HSV-2 gD2 and UL46 and UL47 genes coupled with Vaxfectin® | Intramuscular | Preclinical trial | HSV-2 | Provides protection against lethal HSV-2 challenge | Includes additional controls including irrelevant plasmids coupled with Vaxfectin® | (35) Mackay LK, Wakim L, van Wiet CJ et al. Maintenance of T Cell Function in the Face of Chronic Antigen Stimulation and Repeated Reactivations for a Latent Virus Infection. J Immunol, 186(5), 2173-2178 (2011). |
| Codon-modified polynucleotide vaccine | Intradermal in forearm | Clinical trial | HSV-2 | Reduces viral replication and shedding in genital tract, latent HSV-2 DNA in DRG, and frequency of recurrent disease | Minimal antibodies increase with overall statistical significance | (36) Mackay LK, Stock AT, Ma JZ et al. Chronic Antigen Stimulation and Repeated Shedding of HSV-2 Seronegative Community Residents. J Immunol, 188(12), 5811-5817 (2012). |
| COR-1: (1) Full-length HSV-2 envelope gD2 and (2) truncated version of gD2 fused to a ubiquitin sequence | Intramuscular | Preclinical trial | HSV-2 | Completes protection from both primary and recurrent genital disease | Provides safe and well tolerated vaccine | (37) Masopust D, Picker LJ. Hidden T-cell memories: frontline memory T cells and early protection with no moderate or serious adverse effects |
| SLV-20: (1) pGX27 with tissue plasminogen activator (tPA), FIIIL and HSV-2 gB and UL39, (2) pGX27 with gD2, ICPO and ICP4 and (3) pGX27 with IL-12, IL-21 and MIP-1α | Intramuscular | Preclinical trial | HSV-2 | Presence of CD45+, CD4+, CD68+ macrophages and polymorphonuclear neutrophils at site of immunization | Increases immune cellular activity | (38) Suni MA, Ghanekar SA, Houck DW et al. Long-lived epithelial immunity by tissue-resident memory T (TRM) cells in the absence of persisting local antigen presentation. Proc Natl Acad Sci U S A, 109(18), 7037-7042 (2012). |
| Protein-based subunit vaccine | Intramuscular | Preclinical trial | HSV-1 | Provides protection against acute and recurrent HSV-2 infection | Not as effective as replication-defective d5-29 | (39) Jiang X, Chentou AA, Hsiang C et al. The herpes simplex virus type 1 latency associated transcript (LAT) can protect neuronal derived C1300 and Neuro2A cells from Granzyme B induced apoptosis and CD8+ T-cell killing. J Virol, 84(10), 5241-5247 (2010). |
| lipid A (MPL)- aluminum hydroxide (alum) | Subcutaneous | Preclinical trial | HSV-2 | Provides protection against acute and recurrent HSV infection and acute viral shedding | Does not show any significant differences in immunoglobulin IgA, IgM, IgG1 and IgG3 levels | (40) Mackay LK, Stock AT, Ma JZ et al. Long-lived epithelial immunity by tissue-resident memory T (TRM) cells in the absence of persisting local antigen presentation. Proc Natl Acad Sci U S A, 109(18), 7037-7042 (2012). |
| HSV-2 gD with MPL-alum | Intramuscular | Clinical trial | HSV-1/HSV-2, HSV-1+/HSV-2− | Presents a protective effect in those women who were HSV-1 and HSV-2 seronegative | Not effective in men regardless of serologic status | (41) Jameson SC, Masopust D. Diversity in T cell memory: an embarrassment of riches. Immunity, 31(6), 859-871 (2009). |
| HSV-2 gD and gB adjuvanted with a novel T- cell antigen and tegument protein UL40 | Intramuscular | Preclinical trial | HSV-2 | Increases HSV-2 antigen-specific CD8+ T-cell responses | Does not prevent mucosal infection | (42) Mackay LK, Stock AT, Ma JZ et al. Long-lived epithelial immunity by tissue-resident memory T (TRM) cells in the absence of persisting local antigen presentation. Proc Natl Acad Sci U S A, 109(18), 7037-7042 (2012). |
TABLE 1 | Continued

| Type of Vaccine | Vaccine Construct | Administration Route | Phase(s) | Virus Subtypes | Ref. |
|-----------------|-------------------|---------------------|----------|----------------|------|
| HSV-1 ΔgK vaccine | HSV-1 ΔgK virus is able to encode the ICP0 gene, which is essential for viral genome replication | Intramuscular | Preclinical | HSV-2 | Khan AA et al. Combinatorial HSV Vaccine Strategies (42) Khan AA, Srivastava R, Spencer D |
| HSV-1 ΔgK vaccine | HSV-1 ΔgK virus is able to encode the ICP0 gene, which is essential for viral genome replication | Intramuscular | Preclinical | HSV-2 | Khan AA et al. Combinatorial HSV Vaccine Strategies (42) Khan AA, Srivastava R, Spencer D |
| HSV-1 ΔgK vaccine | HSV-1 ΔgK virus is able to encode the ICP0 gene, which is essential for viral genome replication | Intramuscular | Preclinical | HSV-2 | Khan AA et al. Combinatorial HSV Vaccine Strategies (42) Khan AA, Srivastava R, Spencer D |
| HSV-1 ΔgK vaccine | HSV-1 ΔgK virus is able to encode the ICP0 gene, which is essential for viral genome replication | Intramuscular | Preclinical | HSV-2 | Khan AA et al. Combinatorial HSV Vaccine Strategies (42) Khan AA, Srivastava R, Spencer D |
| HSV-1 ΔgK vaccine | HSV-1 ΔgK virus is able to encode the ICP0 gene, which is essential for viral genome replication | Intramuscular | Preclinical | HSV-2 | Khan AA et al. Combinatorial HSV Vaccine Strategies (42) Khan AA, Srivastava R, Spencer D |
| HSV-1 ΔgK vaccine | HSV-1 ΔgK virus is able to encode the ICP0 gene, which is essential for viral genome replication | Intramuscular | Preclinical | HSV-2 | Khan AA et al. Combinatorial HSV Vaccine Strategies (42) Khan AA, Srivastava R, Spencer D |
| HSV-1 ΔgK vaccine | HSV-1 ΔgK virus is able to encode the ICP0 gene, which is essential for viral genome replication | Intramuscular | Preclinical | HSV-2 | Khan AA et al. Combinatorial HSV Vaccine Strategies (42) Khan AA, Srivastava R, Spencer D |
| HSV-1 ΔgK vaccine | HSV-1 ΔgK virus is able to encode the ICP0 gene, which is essential for viral genome replication | Intramuscular | Preclinical | HSV-2 | Khan AA et al. Combinatorial HSV Vaccine Strategies (42) Khan AA, Srivastava R, Spencer D |
| HSV-1 ΔgK vaccine | HSV-1 ΔgK virus is able to encode the ICP0 gene, which is essential for viral genome replication | Intramuscular | Preclinical | HSV-2 | Khan AA et al. Combinatorial HSV Vaccine Strategies (42) Khan AA, Srivastava R, Spencer D |
| HSV-1 ΔgK vaccine | HSV-1 ΔgK virus is able to encode the ICP0 gene, which is essential for viral genome replication | Intramuscular | Preclinical | HSV-2 | Khan AA et al. Combinatorial HSV Vaccine Strategies (42) Khan AA, Srivastava R, Spencer D |

Replication-Defective HSV Vaccines

Replication-defective virus vaccines, also called DISC (Disabled Infectious Single Cycle) virus vaccines, are defective for one or more genes that are essential for viral genome replication or synthesis and assembly of viral particles. In normal cells, they express viral gene products but do not replicate to form progeny virions. Replication-defective HSV vaccines can stimulate immune responses but produce no progeny viral particles. However, because they do not replicate and spread in the host, replication-defective virus vaccines may be less immunogenic, specifically less T cell stimulators because they have a relatively limited capacity to solicit professional antigen presenting cells (i.e., B, macrophage, and dendritic cells), a prerequisite for the induction of CD4+ and CD8+ T cell responses.

The replication-defective HSV vaccines developed during the last 24 years include: (1) DISC HSV-1 vaccine tested in guinea pigs by McLean, back in 1996 (80); (2) This was followed by another DISC HSV-2 vaccines which consisted of gH-deleted HSV-2 mutant tested in guinea pigs and reported by McLean in 1997 (81); (3) The HSV-2 mutant engineered by Dr. Knipe back in 1997, by replacing the ICP8 gene of HSV-2 strain 186 with an ICP8-lacZ fusion gene from the HSV-1 HD-2 mutant strain. The resulting HSV-2 5BlacZ mutant was later tested in guinea pigs by the same group as reported in 2001 (61, 62), (4) The most studied replication-defective virus HSV-2 dl5-29 vaccine, was developed by Knipe in 2008 and tested in mice and guinea pigs by Cohen in 2010 (12, 59, 63, 82) and by Londono-Hayes in 2015 (14) and shown to be have a protective effect. Eventually, this vaccine progressed to human trials only to show unsuccessful results in a Phase 1 clinical trial conducted recently by Sanofi Pasteur; (5) The HSV-2 ACAM529 mutant tested in a mouse model of genital herpes challenge and reported by Knipe and others in 2010 and 2012 (12, 83, 84); (6) The HSV-1 ΔgK mutant tested in mouse model of herpes challenge and reported in 2013 by Kousoulas (85); (7) The HSV-1 CJ2-gD2 vaccine, a glycoprotein D-expressing replication-defective and dominant-negative HSV-1 recombinant viral vaccine, tested in mice guinea pigs and reported in 2011 (11) and 2014 by Yao (86); (8) The latest replication defective HSV vaccine is the HSV-2 AgD (gD1 del) reported in 2015 by Herold and Jacobs group as being protective in a mouse model of genital herpes challenge (87). The efficacy of the HSV-2 AgD vaccine in prophylactic and therapeutic settings has yet to be evaluated in the guinea pig model of primary and recurrent genital herpes. Compared to clinical trials using adjuvanted subunit vaccines (e.g., the adjuvanted gD/gB nuclear localization signal (NLS) on the ICP0 gene (0DeltaNLS), developed in 2010 by Halford and tested in mice and guinea pigs (69, 73–76); (5) The HSV2-gD27 mutant vaccine reported by Cohen in 2012 (77); (6) The HSV-2 gE2-del mutant vaccine reported by Friedman in 2012 (78); (7) The HSV-2 UL24 mutant tested in mice and guinea pigs reported by Visalli in 2014 (67); and (8) The HSV-1 VC2 mutant reported by Kousoulas in 2014 (79).
vaccine trials), many live attenuated/replication defective vaccines-based Phase 1 trial trials, were either terminated or did not progress to Phase II, because of: (i) A lack of immunogenicity; and/or (ii) Concerns related to safety of using a live virus as vaccine, as detailed above.

Subunit HSV Vaccines

A variety of subunit HSV vaccine approaches have been developed including proteins, DNA and peptide epitope-based vaccines (88, 89). Traditional protein-based vaccines are safe compared to live-attenuated and replication-defective HSV vaccines. Recombinant soluble HSV-2 glycoprotein D (gD) has been the most promising subunit vaccine that went into extensive clinical evaluation. Over the past 25 years, there has been one Phase II therapeutic genital herpes vaccine and three Phase III clinical trials of prophylactic subunit vaccines, all using the HSV-2 gD (or mixed with gB in one trial) (90–95). Back in 1994, the first therapeutic vaccine trial delivered the gD with aluminum salt (i.e. Alum) adjuvant in 98 symptomatic genital herpes patients who reported 4 to 14 recurrences per year (96). Unfortunately, this vaccine reduced the frequency of recurrences by only 24% despite that the vaccine boosted neutralizing antibodies to HSV-2 four-fold over baseline levels (96). These disappointing results from the first therapeutic gD/Alum vaccine trial suggested that for therapeutic protection; a vaccine must: (1) Induce CD4+ and CD8+ T cell responses, in addition to neutralizing antibodies, (2) Incorporate HSV-2 antigens other than gD; and (3) Must test different adjuvants, other than Alum. Three years later in 1997, the Chiron vaccine trial used a combination of gD and gB delivered together with the MF59 Novartis’ adjuvant, an oil-in-water emulsion of squalene oil, using the same target population of genital herpes patients as in the 1994 trial. This gB/gD/MF59 vaccine did not elicit T cell responses, produced high levels of neutralizing antibody to HSV-2, yet had only a 9% efficacy (94). This trial suggested that: (1) besides neutralizing antibodies, a protective vaccine must induce antiviral CD4+ and CD8+ T cell responses; (2) a therapeutic vaccine must incorporate HSV-2 antigens other than gB and gD; and (3) Must test different adjuvants, other than Alum and MF59. Later, two GlaxoSmithKline (GSK) vaccine trials (one reported in 2004 and the other in 2012), used the gD protein delivered together with a different adjuvant, the 3-0-deacetylated monophosphoryl lipid A (MPL), a TLR4 agonist (93) together with Alum (gD/MPL/Alum vaccine). The first trial enrolled discordant couples, who have regular partners with genital herpesis, while the second trial enrolled HSV seronegative women who have multiple and random partners (93). The first trial, reported in 2004, showed a 74% efficacy against genital herpes disease caused by HSV-2 (93). Unfortunately, later, results using the same gD/MPL vaccine reported in 2012, showed only 58% efficacy against genital HSV-2 disease (13). The apparent contradictions in efficacy against genital HSV-2 disease, of the two GSK trials that used the same gD/MPL/Alum vaccine, is puzzling. The difference in efficacy in the two clinical trials attributed to different populations enrolled in each trial (i.e. discordant couples vs. random seropositive women with multiple partners) (13). In the first clinical trial, the distinguishing feature of discordant couples was that they were a highly selected group in which the uninfected partner is potentially repeatedly exposed to HSV by the infected partner. This likely increased risk of infection and disease, hence lowering the threshold of seeing a significant effect of the therapeutic vaccine. In other words, the attack rates of HSV-2 genital disease were high among discordant couples making easy to see a significant reduction following therapeutic vaccination. In contrast, the second clinical trial that enrolled random seropositive women, with multiple lifetime sexual partners, in which the attack rate and the risk of infection and disease was much lower and hence likely raised the threshold of seeing a significant effect of the therapeutic vaccine. Regardless of the targeted population, the first GSK vaccine trial that produced 74% protective efficacy also stimulated both T cells and neutralizing antibodies (13). In 2016-2018, a Genocea vaccine trial (designated as Gen-003) used a combination of ICP4 and gD2 truncated proteins with a novel adjuvant, named Matrix M-2 (MM-2) (89). Matrix M is a saponin-based adjuvant that has a balanced B and T cell immuno-stimulatory profile. This trial reported a significant reduction of recurrent herpes lesions and genital viral shedding (90–92). This protection appeared to correlate with blood-derived antiviral CD4+ and CD8+ T cell responses (90–92). Due to ethical and practical limitations, none of the vaccine clinical trials have investigated the local tissue resident CD4+ and CD8+ T cells in dorsal root ganglia (DRG) and vaginal mucosal tissues.

MODIFIED RNA (MRNA) VACCINE PLATFORMS AGAINST HSV-1 AND HSV-2

RNA vaccines, during the current pandemic, have emerged as a versatile approach against emerging viral infections to overcome the challenges confronted with the conventional vaccine strategies 1–7. mRNA is the carrier of the genetic information necessary for the endogenous proteins synthesis, it does not integrate into the genome and safely metabolized and eliminated by the cells 8–10. RNA-based vaccines have been shown safe in animal models and in human clinical trials and trigger a strong innate immune response. Many strategies have been used to increase the delivery and immunogenicity of mRNA while diminishing innate immune sensing 11. Free and protamine-complexed mRNA were among the first approaches to provide robust antigen expression and immune-stimulation 12–14. This vaccine set-up showed the ability to induce strong immunity and protective efficacy against lethal influenza or rabies viral infections in many animal models 4,15. The first ever prophylactic mRNA-based vaccine (CV7201) in healthy human volunteers was made against rabies. This vaccine was generally safe and led to the induction of neutralizing antibody that waned one year after the first vaccination 8. The success of mRNA vaccines has greatly benefited from the development of lipid- and polymer-based nanoparticles that protect RNA from degradation, enhanced cell uptake and improve delivery to the
translational machinery. Currently, lipid nanoparticles (LNPs) are the most frequently used and effective agents for in vivo delivery of mRNA vaccines.\(^9\)\(^,\)\(^16\)\(^,\)\(^17\). Recently, the Food and Drug Administration (FDA) issued an Emergency Use Authorization (EUA) for the Pfizer-BioNTech COVID-19 (BNT162b2) vaccine (Pfizer, Inc; Philadelphia, Pennsylvania), nucleoside-modified mRNA vaccine formulated lipid nanoparticle-encoding the spike glycoprotein of SARS-CoV-2, the virus that causes coronavirus disease 2019 (COVID-19).\(^19\) This technology has encouraged other groups working on vaccines against cancer and viral pathogens to use the NLP-formulated mRNA platform. Recently, the Friedman group\(^18\) showed that nucleoside-modified mRNA in lipid nanoparticle vaccine encoding for glycoproteins gC, gD, and gE induced strong and protective immunity against acute and latent herpes simplex virus type 2 infection in mice. Indeed, and in a side-by-side experiment they compared two vaccine platforms: (1) Trivalent gC2/gD2/gE purified glycoproteins were given with adjuvants (CpG and Alum)\(^19\) and (2) modified mRNA encoding the 3 glycoproteins formulated in lipid nanoparticles (LNP)\(^20\). The RNA was modified to increase the cellular uptake and prevent the innate immunity sensors from inhibiting the translation machinery.\(^21\) The mRNA-LPN vaccine demonstrated to induce effective T-follicular helper and germinal center B cell responses translated into high titers and durable antibodies responses\(^22\) that outperform the glycoproteins-based vaccine in preventing HSV-1 and HSV-2 genital infection and in protecting mice and guinea pigs against intravaginal HSV-2 infection.\(^20\)

LESSONS LEARNED FROM PAST HSV VACCINE CLINICAL TRIALS

The vaccine clinical trials produced valuable lessons that should help improve future herpes subunit vaccines. Specifically, these trials emphasize four major gaps in our current knowledge: (1) The need to incorporate protective herpes protein Ags, other than gB and gD, in the development of a future herpes therapeutic vaccine; (2) The need to design a vaccine strategy that induces anti-viral CD4\(^+\) and CD8\(^+\) T cell-mediated immunity (in addition to HSV-specific neutralizing antibodies) for a better protection against recurrent herpes. (3) This includes exploring new adjuvants and antigen delivery systems, and (3) The need to develop a mucosal vaccine strategy that would induce strong tissue resident CD4\(^+\) and CD8\(^+\) T\(_{RM}\) cells (beside mucosal antibodies such as IgA) that would reduce virus reactivation from latently infected dorsal root ganglia (DRG) and subsequent virus shedding in the genital tract and recurrent herpetic disease. This is because of the failure of past parenteral subunit vaccines that elicit systemic immune responses against HSV-2. Although most of these vaccine research trials have not been promising, we have gained a better understanding of the correlates of protective immunity for a therapeutic HSV vaccine, forming the platform for novel combinatorial vaccine strategies against HSV.

Phenotypic and Functionally Differential HSV-Specific Memory CD8\(^+\) T Cell Subsets in Asymptomatic and Symptomatic HSV Infected Individuals

Understanding the immune mechanisms by which seropositive asymptomatic individuals are protected from recurrent herpes disease is significantly important as exploiting it can elicit a T cell-based immune response in the mucosa lining the genital tract to prevent HSV acquisition. Recurrent genital herpes disease occurs following periodic reactivation of the virus that travels the axons of DRG neurons to re-infect the genital tract (GT), where lytic replication leads to herpetic lesions and transmission.\(^15\) In asymptomatic individuals (ASYMP) HSV reactivation never causes recurrent disease.\(^16\)\(^–\)\(^18\),\(^20\). In symptomatic individuals (SYM), HSV reactivation often causes painful recurrent genital disease.\(^17\)\(^,\)\(^19\)\(^,\)\(^21\)\(^,\)\(^22\). Reports on HSV therapeutic vaccine trials have shown that both innate and adaptive immunity play an equal role in directing the right immune response to prevent disease by causing a low to no-shedding of the virus. Our research group has explored the differential immune scenarios present in asymptomatic protected individuals that gives them the natural immunity to contain recurrence of herpes. The asymptomatic and symptomatic individuals are strikingly different in their HSV-specific CD8\(^+\) T memory cell immune-profile. After resolution of primary genital herpes infection, a heterogeneous pool (in terms of anatomic distribution, phenotype and fu) of HSV-specific memory CD8\(^+\) T cells develops\(^23\) and can be divided into three major subsets: (1) effector memory CD8\(^+\) T cells (T_EM); (2) central memory CD8\(^+\) T cells (T_CLM); and (3) tissue-resident memory CD8\(^+\) T (T_RM) cells. The different CD8 memory T cell subsets in HSV infection is illustrated in Figure 1. Regarding anatomic distribution, effector memory CD8\(^+\) T_EMs and central memory CD8\(^+\) T_CLM cells circulate between lymphoid and non-lymphoid tissues, such as the DRG and GT (24). The third subset does not enter circulation, but is instead selectively retained in infected tissues, such as DRG (25–27) and GT (25, 28), as a tissue-resident memory CD8\(^+\) T_RM cells. These CD8\(^+\) T_RM cells are poised for immediate response to reactivation from DRG (25, 29) and inhibit virus replication at GT (25). T_RM cells have altered T cell trafficking patterns due to the down-regulation of T cell homing molecules CD62L and CCR7 (30–34). The phenotypic profile of T_CLM cells is CD8CD103\(^{low}\)CD62L\(^{high}\) CCR7\(^{high}\). T_EM cells are CD8\(^+\)CD103\(^{low}\)CD62L\(^{low}\)CCR7\(^{low}\). T_RM cells are CD8\(^+\)CD103\(^{high}\)CD62L\(^{low}\)CCR7\(^{low}\)CD11a\(^{high}\)CD69\(^{high}\) (24, 35, 36). T_EM and T_RM cells, but not T_RM cells, express CD103. T_CLM cells must proliferate and undergo differentiation for effector function (37–40). In contrast, T_EM and T_RM cells are already differentiated and poised for immediate effector function (41). We recently discovered that most HSV-specific CD8\(^+\) T cells from ASYMP individuals expressed low levels of lymphoid homing markers (CD62L\(^{low}\)CCR7\(^{low}\)), suggesting that these T cells are predominantly of a CD8\(^+\) T_EM cell subset. In contrast, most HSV-specific CD8\(^+\) T cells from SYMP individuals are predominantly of T_CLM cell subset (42). Moreover, a decline in the
number and function of memory CD8+ T cells positively correlated with severe recurrent genital disease in SYMP individuals. The critical role of antigen-specific CD8+ T cells has been demonstrated in studies using various animal models (43, 44). We are now beginning to appreciate the differences observed in CD8 T cell memory population in symptomatic and asymptomatic HSV infected individuals, and understand the importance of stimulating tissue-resident memory T cells for prevention of HSV infection in the mouse model (44). T cell-based immunotherapeutic strategies to treat recurrent herpes infection and disease are emerging for HSV, and our laboratory has contributed significantly towards developing human asymptomatic CD8+ T cell epitopes for HSV immunotherapy (20, 44, 97, 98). In the last fifteen years of vaccine development, we have succeeded in identifying new HLA-A2*01 restricted "asymptomatic" human CD4+ and CD8+ T cell epitopes from HSV-1 gB and gD glycoproteins and from HSV-1 VP11/12 and VP13/14 tegument proteins. Ocular herpes models using HLA-A2*01 restricted transgenic mouse and rabbits have shown that these asymptomatic human epitopes stimulated protective CD8 T cell responses (21, 99, 100). Presently, we are making significant headway with novel combinatorial approaches to use these epitopes as a SAPN (self-assembling protein nanoparticle) with built-in flagellin domains as a therapeutic HSV vaccine.

**PRIME AND PULL VACCINES USING ADENOVIRAL VECTORS DELIVERING EPITOPES TOGETHER WITH T-CELL CHEMOKINES INTO HSV INFECTED TISSUES**

Chemokines are naturally produced by our immune system and could serve as safer and reliable adjuvants (101). Memory CD8+ T cells specific for HSV play an important role in inhibiting HSV-1 reactivation from TG and subsequent viral shedding in tears that trigger the recurrent corneal herpetic disease. The CXC chemokine ligand 10 (CXCL10)/CXC chemokine receptor 3 (CXCR3) pathways are critical in promoting T cell immunity against many viral infections (102). In a "prime and pull" strategy, a topical chemokine was applied to the genital mucosa after subcutaneous vaccination to pull HSV-specific CD8+ T cells and was shown to be associated with decreased...
disease upon challenge with HSV-2 (103). The CXCL10/CXCR3 pathway also affects TG- and cornea-resident CD8+ T cell responses to recurrent ocular herpes virus infection and disease (104). Chemokines can also be co-delivered in a DNA vaccine for immunomodulation. Adenovirus-CCL21 transduced class I peptide-pulsed DC, and autologous DC-adenovirus CCL21 vaccines are currently in Phase I clinical trials for the treatment of malignant melanoma and stage IIIB-IV or recurrent non-small lung cancer respectively while XCL1 along with the IL-2 gene (CHESAT tumor vaccine) is in a clinical trial for neuroblastoma (101). Pre-clinical studies in HSV have shown immuno-potentiation of DNA vaccines by co-delivery of chemokines such as CCR7 ligands and IL-8, RANTES delivered to the mucosa (105, 106). We are in the advent of testing multi-epitope vaccine that co-delivers chemokines using adenovirus vectors. A “Prime-Pull-Keep” Therapeutic Vaccine (PPK Vaccine) is being designed to boost Neutralizing IgG/IgA induced viral reactivation in explanted mouse sensory ganglia (118, 119) and may similarly reduce detectable HSV-1 and HSV-2 reactivation in vivo (120–123). During acute (11 days) and latent (30 days) post-infection HSV-1 infection of mice, most effector CD8+ T cells from sensory ganglia simultaneously express high levels of 2 to 3 immune checkpoint receptors (e.g. PD-1 and LAG-3) (39, 111, 116, 117). This phenotype correlated with functional exhaustion of HSV-specific CD8+ T cells in symptomatic individuals with increased virus titers and severe disease. In mice, like humans, HSV-1 latently infected sensory ganglia have chronic CD8+ T cell infiltrates (118). HSV-specific CD8+ T cells producing IFN-γ and Granzyme B appear to suppress (or abort) increased virus reactivation from infected sensory ganglia explants (39, 111, 116, 117). This phenotype correlated with functional exhaustion of sensory ganglia-derived CD8+ T cells and increased virus reactivation from infected sensory ganglia explants (39, 111, 116, 117).

**Laser Adjuvants**

As an alternative to currently used conventional adjuvants, the chemical- and biological-free laser-adjuvant offers a well-tolerated, simple to produce method to enhance mass vaccination for widespread viral infections (107). Studies from our laboratory have reported that skin exposure of B6 mice with the FDA approved non-ablative fractional diode laser (PalooVia Laser), followed by an intradermal delivery of a HSV peptide vaccine, safely induced potent and sustained HSV-specific CD8+ T cells, detected in both the draining lymph nodes (DLN) and in the vaginal mucosa (VM) (108). In the vaginal mucosa of laser-treated and peptide vaccinated mice, we observed more HSV-specific effector memory CD8+ T cells. Following an intravaginal HSV-2 challenge, we found decreased genital herpes lesions and increased DC infiltrates around the laser-treated skin area. These findings have important implications for the development of efficient vaccine immunization strategies against HSV-1 and HSV-2.

**IMMUNE CHECKPOINT BLOCKADE COMBINED WITH THERAPEUTIC HERPES VACCINE**

Total or partial loss of T cell function (dysfunction) occurs following repetitive HSV latent/reactivation cycles (109–111) and exposure to antigens is termed exhaustion (112) and is usually linked with expression of T cell co-inhibitory receptors: PD-1, TIM-3, LAG-3 (CD223), TIGIT, PSGL-1, 2B4 (CD244), GITR, CTLA-4 (CD152), CD160, and BTLA (CD272) (113, 114). T cell dysfunction requires two signals: (1) T cell receptor (TCR) engaged by MHC presenting an HSV epitope (113); and a (2) T cell co-inhibitory receptor (e.g., PD-1) engaged by ligand (i.e., PDL-1). In humans, latent HSV in sensory ganglia is accompanied by chronic CD8+ T cell infiltrates (115). A portion of viral reactivation in sensory ganglia appears to be controlled by CD8+ T cell-mediated mechanisms (111, 116, 117). Recently, we compared the expression levels of eight known T cell co-inhibitory receptors on blood-derived HSV-specific CD8+ T cells from symptomatic and asymptomatic HSV infected individuals and discovered that, HSV-specific CD8+ T cells from symptomatic individuals expressed significantly higher levels of T cell co-inhibitory receptors like PD-1, LAG-3, TIM-3 and TIGIT (Figure 1). This phenotype correlated with functional exhaustion of HSV-specific CD8+ T cells in symptomatic individuals with increased virus titers and severe disease. In mice, like humans, HSV-1 latently infected sensory ganglia have chronic CD8+ T cell infiltrates (118). HSV-specific CD8+ T cells producing IFN-γ and Granzyme B appear to suppress (or abort) induced viral reactivation in explanted mouse sensory ganglia (118, 119) and may similarly reduce detectable HSV-1 and HSV-2 reactivation in vivo (120–123). During acute (11 days) and latent (30 days) post-infection HSV-1 infection of mice, most effector CD8+ T cells from sensory ganglia simultaneously express high levels of 2 to 3 immune checkpoint receptors (e.g. PD-1 and LAG-3) (39, 111, 116, 117). This phenotype correlated with functional exhaustion of sensory ganglia-derived CD8+ T cells and increased virus reactivation from infected sensory ganglia explants (39, 111, 116, 117).

**HERPES VACCINE- SAFETY EVALUATION**

Safety concerns for vaccines include: (i) the potential inherent toxicities of the antigen and the adjuvants, as well as potential toxicities due to interactions of the components present in the final formulation; and (ii) the possibility that the vaccine induces inflammatory responses that may lead to undesired toxic side effects. Some adjuvants may elicit elevated levels of
proinflammatory cytokines and other mediators of toxicity, irrespective of the immune response against the antigen. Preclinical standard repeated-dose toxicology studies performed in animals will identify whether intrinsic toxicity and immunotoxicity are: (i) confined primarily to the sites of injection; (ii) caused by the delivery method (i.e., the side effects are seen in both control and vaccinated animals) or (iii) caused by the intended immune responses to the vaccine (i.e., side effects occur with greater frequency and severity in vaccinated animals compared to controls). (1) Parameters for monitoring of systemic toxicity: Toxicity studies, repeated-dose toxicity studies, address the potential for systemic toxicity including, but not limited to, the systemic effects on the immune system. A broad spectrum of information should be obtained from the toxicity study, and both in-life and postmortem data should be collected. This routinely includes careful monitoring of body weight and food consumption, body temperature, histopathology, clinical chemistry, hematology, coagulation parameters and acute phase reactants. (2) Parameters for monitoring of local reactogenicity: Local toxicity studies of intramuscularly administered vaccines should preferably be conducted in animals with sufficient muscle mass, (such as rabbits) to test the full human dose of the final vaccine formulation.

CONCLUSIONS

Since most of the current HSV vaccine candidates were not promising individually in clinical trials, combinatorial vaccine approach seems to be the most appropriate in the present scenario to further advance HSV vaccine trials. Combinatorial application practically poses many problems and hence requires optimization in animal models. For example, one such approach optimized in the guinea pig model in our laboratory, is illustrated in Figure 1.

Results from clinical trials of the HSV vaccine indicate that it is essential to explore combinatorial approaches in the discovery of an effective therapeutic vaccine. Our long-term goal is to develop a long-lasting immunotherapeutic vaccine against genital herpes. HSV-specific CD8+ T cells are critical in preventing HSV reactivations from neurons of DRG and in limiting the severity of GT inflammatory lesions by reducing HSV replication (138–142). By harnessing the immune mechanisms active in seropositive asymptomatic individuals that make them “naturally” protected from recurrent herpes disease, we came up with a multiple-asymptomatic/protective epitope-based vaccine strategy, a promising HSV vaccine candidate when combined with other T cell-based immunotherapies like immune-checkpoint blockade or immunomodulation using various chemokines.

EXPERT REVIEW

- The latest failures of most of the clinical herpes vaccines indicate that immunotherapeutic vaccine against HSV should be efficient in eliciting antigen-specific immune responses that contain reactivation of the virus, to control both recurrent lesions and viral shedding. Our vaccine research approach is based on the understanding and harnessing of immune strategies that make the seropositive asymptomatic individuals “naturally” protected from recurrent herpes disease throughout their life. We realized that the best strategy for an effective HSV vaccine would be to elicit a T cell-based immune response that boosts HSV specific effector memory T cell functionalities in the mucosal lining to prevent HSV-1/HSV-2 acquisition/reactivation.

- Much remains unknown about the protective immune effector of herpes, however, improved knowledge of HSV immunology, and immunopathology should help guide new vaccine strategies for HSV. In the last fifteen years of vaccine development, we have succeeded in identifying many protective “asymptomatic” human CD4+ and CD8+ T cell epitopes from HSV-1 gB and gD glycoproteins and from HSV-1 VP11/12 and VP13/14 tegument proteins. We are currently progressing with novel combinatorial approaches to use these epitopes as a SAPN with built-in flagellin domains as therapeutic HSV vaccine. A Prime-Pull-Keep Therapeutic Vaccine (PPK Vaccine) is designed to boost Neutralizing IgG/IgA antibodies (Abs) and boost the number and function of antiviral CD4+ and CD8+ T RM cells within the cervico genital mucocutaneous (CGMC) and dorsal root ganglia (DRG) tissues. PPK vaccine is expected to help STOP the virus reactivation from latently infected DRG, virus shedding and virus replication in CGMC, thus curing or reducing recurrent genital herpes disease.

- Since most of the current HSV vaccine candidates were not promising individually in clinical trials, a combinatorial vaccine approach seems to be the most appropriate in the present scenario to further advance HSV vaccine trials. Combinatorial application practically poses many problems and hence requires optimization. We are currently optimizing these combinatorial approaches in animal models. We came up with multiple-asymptomatic/protective epitope-based vaccine strategy which will be a promising HSV vaccine candidate when combined with other T cell-based immunotherapy-like immune-checkpoint blockade or immunomodulation using various chemokines.

AUTHOR CONTRIBUTIONS

AC, ND, RS, SP, P-GC, and LB: conceived and designed the experiment, performed the experiments, contributed reagents, materials, and analysis tools. AC, ND, RS, SP, P-GC, LZ, HV, HC, KH-C, and LB wrote the paper. All authors contributed to the article and approved the submitted version.

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