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Future of an “Asymptomatic” T-cell Epitope-Based Therapeutic Herpes Simplex Vaccine

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Summary

Considering the limited success of the recent herpes clinical vaccine trial [1], new vaccine strategies are needed. Infections with herpes simplex virus type 1 and type 2 (HSV-1 & HSV-2) in the majority of men and women are usually asymptomatic and results in lifelong viral latency in neurons of sensory ganglia (SG). However, in a minority of men and women HSV spontaneous reactivation can cause recurrent disease (i.e., symptomatic individuals). Our recent findings show that T cells from symptomatic and asymptomatic men and women (i.e. those with and without recurrences, respectively) recognize different herpes epitopes. This finding breaks new ground and opens new doors to assess a new vaccine strategy: mucosal immunization with HSV-1 & HSV-2 epitopes that induce strong in vitro CD4 and CD8 T cell responses from PBMC derived from asymptomatic men and women (designated here as “asymptomatic” protective epitopes”) could boost local and systemic “natural” protective immunity, induced by wild-type infection. Here we highlight the rationale and the future of our emerging “asymptomatic” T cell epitope-based mucosal vaccine strategy to decrease recurrent herpetic disease.

Key terms

HSV-1; HSV-2; Herpes; T cells; Antigen; Epitope; Human; Symptomatic; and Asymptomatic; Vaccine; Therapeutic

Immuno-biology of herpes

Herpes simplex viruses type 1 and type 2 (HSV-1 & HSV-2) are infectious pathogens that cause serious diseases at every stage of life including fatal disseminated disease in newborns, to cold sores, genital ulcerations, eye disease, and fatal encephalitis in adults [2–6]. Recurrent ocular herpes infection (primarily by HSV-1) causes herpetic stromal keratitis (HSK), an immunopathological lesion of the cornea that can lead to blindness [7–11]. More than 450,000 people in the United States have a history of recurrent ocular herpes disease capable of causing loss of vision [7, 8, 12, 13], and over 100 million individuals are infected with HSV-1 [14]. HSV-1 is also primarily responsible for oro-facial herpes, also known as

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oro-labial herpes, described as an eruption of painful sores in and around the mouth [15, 16]. Recurrent genital herpes infection (primarily by HSV-2) also leads to an immunopathological response that produces genital ulcerations and scarring [10, 11]. The prevalence of HSV-2 seropositive individuals at the age of 15 years and older, is estimated to be at least 45 million within the United States [17, 18] and well over 530 million worldwide, with a greater frequency of infection in women [19–21]. 30 to 60 percent of women receiving obstetric care in the United States are seropositive for HSV-1 and/or HSV-2, with their newborns particularly susceptible to neonatal infection, permanent brain damage, birth defects and death [22–24].

The recent “failure” of genital herpes vaccine trials [1], sets an open challenge and should motivate researchers to overcome this hurdle. In this vein, a novel “asymptomatic” herpes vaccine approach has recently been initiated. This strategy is based on characterizing the “symptomatic” and “asymptomatic” human T cell epitopes on HSV target antigens and selectively including those asymptomatic epitopes in the vaccine construct instead of using the full length of target antigen. The “symptomatic” epitopes, which may be harmful, are excluded from the asymptomatic vaccine. This novel approach is expected to improve the protective efficacy as well as eliminate the pathogenic effect of the vaccine.

Latency and reactivation

During primary HSV infection, virions are transported by retrograde flow along axons that connect the point of entry into the body to the nuclei of sensory neurons [25–29]. Viral replication occurs in a small number of sensory neurons, and the viral genome then remains in a non-reproductive latent state for the life of the host. The HSV-1 Latency-Associated Transcript (LAT) gene, the only viral gene abundantly transcribed during latent infection, promotes survival of infected sensory neurons by reducing apoptosis, thus keeping the virus in the immune sanctuary of neurons [30]. Recently we demonstrated that LAT functions as an immune evasion gene by inducing exhaustion of HSV-specific CD8⁺ T cells [28] and interfering with phenotypic and functional maturation of dendritic cells [29]. With periodic reactivation brought on by events such as physical, chemical or emotional stress, fever, UV light, and tissue damage, the virus is transported back down the axon to replicate again at or near the original point of entry into the body. Such reactivation can result in clinically apparent disease (lesions) or clinically inapparent (asymptomatic, or subclinical) infection. The mechanisms by which HSV establishes latency are being intensely investigated but remain incompletely understood [28, 29].

Symptomatic and asymptomatic herpes

Shedding of reactivated HSV-1 and HSV-2 leads to clinically apparent recurrent herpetic disease (lesions) and is estimated to occur at rates of 3 to 28 percent in adults who harbor latent virus in their sensory neurons [20, 21, 31–35]. Recurrent disease ranges from rare episodes occurring once every 5–10 years, to outbreaks occurring monthly or even more frequently among a small proportion of “symptomatic patients” [20, 21, 31, 36]. For simplicity, one can categorize seropositive individuals based on the frequency of their recurrent disease into two major groups: (1) symptomatic individuals (with a history of recurrent corneal, genital and/or oro-facial herpetic disease); and (2) asymptomatic individuals (no history of any recurrent herpes disease at any location). Most seropositive individuals do not experience recurrent herpetic disease and are designated “asymptomatic” [21, 31, 34, 36, 37]. In contrast, in “symptomatic” individuals reactivation of latent virus can result in mild to severe herpetic disease [21, 31, 34, 36, 38]. It is not known why HSV-1 and

HSV-2 reactivation/shedding is asymptomatic in some individuals and symptomatic in others, or why the frequency and severity of recurrent disease varies among symptomatic individuals. Interestingly, some studies conclude that for genital herpes, symptomatic and asymptomatic patients shed virus (spontaneous reactivation) at similar rates [34, 39]. It is likely to be the same for ocular herpes, since shedding rates in tears of asymptomatic individuals has been reported to be as high as 33.5% [36, 37, 40, 41]. In other studies, the rate of virus shedding in symptomatic individuals just prior to the appearance of recurrent disease appeared elevated compared to asymptomatic individuals, while between recurrent disease episodes the rate of virus shedding was similar in symptomatic and asymptomatic individuals [34, 39]. Thus, it is possible that in some instances increased virus reactivation as judged by virus shedding, may lead to recurrent disease. The immune mechanism(s) by which asymptomatic patients control herpetic disease and symptomatic patients do not remains to be fully elucidated [42]. Identifying these mechanisms, or at least the viral antigens (Ags) and epitopes involved, is critical to understanding how to protect against recurrent herpetic disease and for rational advances in therapeutic vaccine development.

Past pursuit of herpes simplex vaccines

Many therapeutic herpes simplex vaccines have been pursued in the last 8 decades (reviewed in [21]). In spite of several past clinical trials (reviewed in [21]), that started as early as the 1920's, no herpes simplex vaccine has been proven sufficiently safe and efficient to warrant commercial development. Inactivated virus prepared from formalin treated tissues of HSV-infected animals [43–45] and heat or UV-inactivated HSV grown in embryonic eggs [44, 46] were among the first attempted herpes vaccines. More recent herpes vaccine strategies include replication-defective virus; avirulent HSV mutants lacking ICP8, ICP10, dl5-29 or VHS genes; discontinuously replicating virus known as “disabled infectious single cycle” or “DISC”; a virus with a deletion of UL22 (glycoprotein H) [47]; a non-replicating HSV-2 vaccine that lacks thymidine kinase (HSV-2 TK⁽⁻⁾) [49, 54–56]; RAV 9395 [58], a live-attenuated HSV-2 strain G containing deletions of the virulence genes γ_1 34.5, UL55, and UL56 [58]; attenuated live HSV vaccine strain R7020 [59], deleted for UL54 (ICP27) through the promoter region of ICP4; AD472, an attenuated virus deleted for the γ_1 34.5 gene, UL55-56, UL43.5, and the US10–12 regions of HSV-2 strain G [60]; dl5-29, a replication-defective virus also known as ACAM-529 [61–66]; and a promising live-attenuated vaccine containing an altered ICP0 [67–69]. A number of trials have also used recombinant live-attenuated adenovirus and vaccinia viruses expressing HSV protein antigens [70–72]. Other vaccine strategies include naked DNA plasmids [73], virus-like particles (VLP) [75], and recombinant *Listeria monocytogens* [76, 77] expressing a herpes T cell epitope. Unfortunately, the above vaccines have either not reached phase II clinical trials or have failed to provide significant efficacy in humans.

To reduce safety concerns associated with whole HSV-based or live vector immunizations [78], several mainly protein-in-adjuvant vaccines have been introduced in the last two decades [4, 8, 78–81]. Unfortunately, clinical trials using protein-in-adjuvant HSV-2 vaccines delivered parenterally in women of sero-discordant couples (in which one partner had the virus and the other did not) have shown limited success against genital herpes [1, 26, 78]. Recently, the same HSV-2 glycoprotein gD based vaccine was assessed in more than 8000 women who were seronegative for both HSV-1 and HSV-2. No protection was seen against HSV-2 disease or infection, although protection was seen against HSV-1 genital disease and infection [1]. In both studies good neutralizing antibody responses were elicited [1, 78]. These clinical trials [1, 78], together with our recently reported pre-clinical study using an established murine model of intravaginal immunization [82], suggest the importance of a vaccine that elevates T cell-based, rather than neutralizing antibody, immune responses [83–85]. Thus, T cells appeared to be an important part of naturally

acquired protective immune responses against herpetic disease, and boosting “asymptomatic” T cells by vaccination has dominated much of our research effort.

T-cell-inducing herpes simplex vaccines – what’s the future

Results with the above vaccine strategies have shown that cellular immunity, rather than humoral immunity, appears crucial for protection against herpes. A potential shortcoming of the above whole protein or whole virus vaccine strategies for herpes simplex virus is that they contain symptomatic as well as asymptomatic epitopes (discussed below). It is likely that Ag exposure during long-term herpes simplex infections may shape different T cell repertoires over time, in symptomatic and asymptomatic individuals. The unique epitope-specific T cell repertoire of each symptomatic and asymptomatic individual is thought to regulate whether herpes reactivation will result in viral control, asymptomatic persistence, or severe disease. Thus, in symptomatic individuals, reactivation of latent virus leads to induction of ineffective or “symptomatic” HSV-specific CD4⁺ and CD8⁺ T cells [34, 36, 38]. In contrast, in asymptomatic individuals, reactivation of latent virus leads to induction of protective or “asymptomatic” HSV-specific CD4⁺ and CD8⁺ T cells and subsequent virus control [34, 36, 38].

Our recent findings support that symptomatic and asymptomatic individuals have different levels of HSV-specific T cell repertoires ([42, 89–91], Dervillez, submitted). We found that T cells from symptomatic and asymptomatic individuals, with similar HLA, have dramatically different profiles of responses to HSV epitopes. A set of human T cell epitopes from HSV-1 glycoproteins B and D (gB & gD) are strongly recognized by T-cells from HSV-1 seropositive asymptomatic individuals, but only weakly by T-cells from symptomatic individuals [42, 89–91]. In contrast, a different, non-overlapping set of gB and gD epitopes are strongly recognized by T-cells from symptomatic but not by T-cells from asymptomatic individuals. However, these differences are not due to clonal T cell deletion since there is not a complete lack of T cell response. The “asymptomatic” T cell precursor appears to exist in symptomatic patients and vice versa. Based on these findings, we hypothesize that a vaccine containing symptomatic epitopes may induce immunopathologic responses and thus mask protection induced by asymptomatic epitopes contained in the vaccine. This may explain why whole protein and whole virus vaccine strategies are not protective against herpes simplex virus. We therefore propose that the vaccine should contain only asymptomatic epitopes. This requires an epitope based vaccine approach.

Future therapeutic “asymptomatic” herpes vaccines

A good starting point for the development of an efficient therapeutic herpes vaccine would be to identify the matrices of protective “asymptomatic” Ags and epitopes strongly recognized by T cells from asymptomatic individuals (as illustrated in Fig. 1). Our pre-clinical vaccine trial in HLA transgenic (HLA Tg) rabbits showed that immunization with asymptomatic human CD8⁺ T-cell epitopes from HSV-1 gD, induced strong human epitope-specific CD8⁺ T cell responses, reduced HSV-1 shedding in tears and reduced corneal disease following an ocular challenge [2]. Rabbits have spontaneous reactivation of HSV-1 similar to humans (~10%). The rate of recurrent corneal disease in rabbits is also similar to that of humans. Our preliminary study suggests that HSV infected HLA transgenic rabbits that developed recurrent corneal disease (i.e., a “symptomatic” HLA transgenic rabbit) are able to respond to asymptomatic epitope vaccination (BenMohamed, unpublished data). This suggests that symptomatic individuals will be able to respond appropriately to a therapeutic asymptomatic epitope based vaccine and develop a CD8 T-cell response specific to the asymptomatic epitopes in the vaccine. In contrast, a therapeutic vaccine containing whole virus or whole viral proteins would be expected to induce symptomatic as well as

asymptomatic CD8 T-cell responses, thus boosting harmful as well as protective immunity. Obviously, boosting harmful immunity should be avoided. This can be accomplished using an asymptomatic epitope based therapeutic vaccine.

Future perspective

Over the last four decades, the field of herpes simplex virus vaccine has progressed rapidly. The safety, immunogenicity and protective efficacy of many vaccine strategies have been assessed pre-clinically and clinically, leading to an appreciation that induction of cellular immunity is crucial for protection. A new vaccine strategy, that uses a mucosal vaccine based on “asymptomatic” T cell epitopes, can boost both local mucosal and systemic protective immunity in those individuals that are already infected. The identification and characterization of the spectrum of HSV-1 and HSV-2 epitopes recognized by T cells from asymptomatic and symptomatic patients, a relatively novel approach we have recently introduced [31, 42, 90], should break new ground in our understanding of the immune mechanisms underlying herpes disease and may ultimately lead to an effective vaccine. A multitude of complex cellular and molecular mechanisms underlying the protective efficacy vs. pathology of “asymptomatic” and “symptomatic” T cell epitopes may be in play: (1) The pathogenic “symptomatic” epitopes may direct the T cell responses away from those that are best suited to clear the viral infection with minimal pathology. (2) An immunopathogenic “symptomatic” response might occur through stimulating low-affinity oligoclonal responses that inhibit broad-based high-affinity responses to other well-presented epitopes; thus deviating protective cytokine responses to damaging cytokine responses. (3) T cell cross-reactivity with epitopes from other viruses, within or outside the herpes family, can also play roles in protective heterologous immunity vs. damaging heterologous immunopathology, as has been reported in other systems [92, 93]. (4) The precursor frequency, proliferative capacity, and functional properties of epitope-specific “symptomatic” and “asymptomatic” T cells in a given individual might also be a factor. Indeed, the T cell repertoire of individuals with the same MHC restriction elements can vary significantly because of “heterologous immunity” and “private specificity” (5). There might also be differences in T cell infiltration/homing to sites of infection induced by “symptomatic” vs. “asymptomatic” epitopes. (6) “Asymptomatic epitopes” might trigger proliferation of “protective” T cells. Inversely, “symptomatic” epitopes might trigger proliferation of “pathogenic” T cells. The use of a human leukocyte antigen (HLA) transgenic rabbit model that (1) develops spontaneous HSV-1 reactivation; (2) develops recurrent ocular herpes disease, and (3) can mount a herpes specific T-cell response to HLA-restricted human T-cell epitopes, is expected to solve many problems in, and speed up development of, herpes vaccines for humans. Finally, we have recently found that there is significant exhaustion of HSV-1 specific CD8⁺ T-cells in sensory ganglia in mice during latency [28, 29]. This condition may well reduce the efficacy of CD8⁺ T cells in controlling reactivation in neurons. Therefore, it may be useful to complement the therapeutic asymptomatic epitope-based vaccine strategy by blocking the exhaustion-pathway. This is likely to result in an even stronger protective CD8⁺ T cell response in latently infected “symptomatic” individuals.

BIBLIOGRAPHY

1. Belshe, Pb; Leone, Pa; Bernstein, Di, et al. Efficacy Results of a Trial of a Herpes Simplex Vaccine. *N Engl J Med.* 2012; 366:34–43. The latest protein-based herpes vaccine that failed in clinical trial. [PubMed: 22216840]
2. Chentoufi, Aa; Dasgupta, G.; Christensen, Nd, et al. A novel HLA (HLA-A*0201) transgenic rabbit model for preclinical evaluation of human CD8⁺ T cell epitope-based vaccines against ocular herpes. *J Immunol.* 2010; 184(5):2561–2571. A first pre-clinical trial of an asymptomatic herpes vaccine in an HLA transgenic rabbit model. [PubMed: 20124097]

3. Chentoufi, Aa; Dasgupta, G.; Nesburn, Ab, et al. Nasolacrimal duct closure modulates ocular mucosal and systemic CD4(+) T-cell responses induced following topical ocular or intranasal immunization. *Clin Vaccine Immunol.* 2010; 17(3):342–353. [PubMed: 20089796]
4. Dasgupta G, Nesburn Ab, Wechsler Sl, BenMohamed L. Developing an asymptomatic mucosal herpes vaccine: the present and the future. *Future Microbiol.* 2010; 5(1):1–4. [PubMed: 20020824]
5. Whitley, Rj; Miller, Rl. Immunologic approach to herpes simplex virus. *Viral Immunol.* 2001; 14(2):111–118. [PubMed: 11398806]
6. Whitley, Rj; Roizman, B. Herpes simplex virus infections. *Lancet.* 2001; 357(9267):1513–1518. [PubMed: 11377626]
7. BenMohamed L, Bertrand G, Mcnamara Cd, et al. Identification of novel immunodominant CD4+ Th1-type T-cell peptide epitopes from herpes simplex virus glycoprotein D that confer protective immunity. *J Virol.* 2003; 77(17):9463–9473. [PubMed: 12915561]
8. BenMohamed L, Wechsler Sl, Nesburn Ab. Lipopeptide vaccines--yesterday, today, and tomorrow. *Lancet Infect Dis.* 2002; 2(7):425–431. [PubMed: 12127354]
9. Zhang X, Issagholian A, Berg Ea, Fishman Jb, Nesburn Ab, Benmohamed L. Th-cytotoxic T-lymphocyte chimeric epitopes extended by Nepsilon-palmitoyl lysines induce herpes simplex virus type 1-specific effector CD8+ Tc1 responses and protect against ocular infection. *J Virol.* 2005; 79(24):15289–15301. [PubMed: 16306600]
10. Bettahi I, Zhang X, Afifi Re, Benmohamed L. Protective immunity to genital herpes simplex virus type 1 and type 2 provided by self-adjuvanting lipopeptides that drive dendritic cell maturation and elicit a polarized Th1 immune response. *Viral Immunol.* 2006; 19(2):220–236. [PubMed: 16817765]
11. Nesburn, Ab; Bettahi, I.; Zhang, X., et al. Topical/mucosal delivery of sub-unit vaccines that stimulate the ocular mucosal immune system. *Ocul Surf.* 2006; 4(4):178–187. [PubMed: 17146573]
12. Dana, Mr; Qian, Y.; Hamrah, P. Twenty-five-year panorama of corneal immunology. emerging concepts in the immunopathogenesis of microbial keratitis, peripheral ulcerative keratitis, and corneal transplant rejection. *Cornea.* 2000; 19(5):625–643. [PubMed: 11009315]
13. Smith, Js; Herrero, R.; Bosetti, C., et al. Herpes simplex virus-2 as a human papillomavirus cofactor in the etiology of invasive cervical cancer. *J Natl Cancer Inst.* 2002; 94(21):1604–1613. [PubMed: 12419786]
14. Whitley, Rj; Roizman, B. Herpes simplex viruses: is a vaccine tenable? *The Journal of clinical investigation.* 2002; 110(2):145–151. [PubMed: 12122103]
15. Guillaume, Jc. Antiviral and non-antiviral general treatments for oro-facial and genital herpes (pregnancy and neonates excluded). *Ann Dermatol Venereol.* 2002; 129(4 Pt 2):625–634. [PubMed: 12122333]
16. Kriesel, Jd; Jones, Bb; Matsunami, N., et al. C21orf91 genotypes correlate with herpes simplex labialis (cold sore) frequency: description of a cold sore susceptibility gene. *J Infect Dis.* 2011; 204(11):1654–1662. [PubMed: 22039568]
17. Xu F, Sternberg Mr, Kottiri Bj, et al. Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. *JAMA.* 2006; 296(8):964–973. [PubMed: 16926356]
18. Fleming, Dt; Mcquillan, Gm; Johnson, Re, et al. Herpes simplex virus type 2 in the United States, 1976 to 1994. *N Engl J Med.* 1997; 337(16):1105–1111. [PubMed: 9329932]
19. Looker, Kj; Garnett, Gp; Schmid, Gp. An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection. *Bull World Health Organ.* 2008; 86(10):805–812A. [PubMed: 18949218]
20. Kalantari-Dehaghi M, Chun S, Chentoufi Aa, et al. Discovery of potential diagnostic and vaccine antigens in herpes simplex virus-1 and -2 by proteome-wide antibody profiling. *J Virology.* 2012 In press.
21. Chentoufi, Aa; Kritzer, EDYm; Nesburn, Ab; Benmohamed, L. Towards a Rational Design of an Asymptomatic Clinical Herpes Vaccine: The Old, The New, and The Unknown. *Clinical and Developmental Immunology.* 2012 In Press. A first discovery of human symptomatic and asymptomatic epitopes in herpes glycoprotein B (gB).

22. Kimberlin, Dw; Whitley, Rj; Wan, W., et al. Oral acyclovir suppression and neurodevelopment after neonatal herpes. *N Engl J Med.* 2011; 365(14):1284–1292. [PubMed: 21991950]
23. Brown, Za; Gardella, C.; Wald, A.; Morrow, Ra; Corey, L. Genital herpes complicating pregnancy. *Obstet Gynecol.* 2005; 106(4):845–856. [PubMed: 16199646]
24. Corey L, Wald A. Maternal and neonatal herpes simplex virus infections. *N Engl J Med.* 2009; 361(14):1376–1385. [PubMed: 19797284]
25. Kimberlin, Dw; Rouse, Dj. Clinical practice. Genital herpes. *N Engl J Med.* 2004; 350(19):1970–1977. [PubMed: 15128897]
26. Dasgupta G, BenMohamed L. Of mice and not humans: how reliable are animal models for evaluation of herpes CD8(+)-T cell-epitopes-based immunotherapeutic vaccine candidates? *Vaccine.* 2011; 29(35):5824–5836. An overview of how animal models are important in developing a therapeutic vaccine against herpes. [PubMed: 21718746]
27. Sullivan-Bolyai, Jz; Hull, Hf; Wilson, C.; Smith, Al; Corey, L. Presentation of neonatal herpes simplex virus infections: implications for a change in therapeutic strategy. *Pediatr Infect Dis.* 1986; 5(3):309–314. [PubMed: 3014452]
28. Chentoufi, Aa; Kritzer, E.; Tran, Mv, et al. The herpes simplex virus 1 latency-associated transcript promotes functional exhaustion of virus-specific CD8+ T cells in latently infected trigeminal ganglia: a novel immune evasion mechanism. *J Virol.* 2011; 85(17):9127–9138. A first demonstration of an immune evasion function of HSV-1 LAT gene. [PubMed: 21715478]
29. Chentoufi, Aa; Dervillez, X.; Dasgupta, G., et al. The Herpes Simplex Virus Type 1 Latency Associated Transcript Inhibits Phenotypic and Functional Maturation of Dendritic Cells. *Viral Immunology.* 2012 In Press. A first demonstration that HSV-1 LAT gene interferes with the function of dendritic cells and hence of CD8 T cell immunity.
30. Perng, Gc; Jones, C.; Ciacci-Zanella, J., et al. Virus-induced neuronal apoptosis blocked by the herpes simplex virus latency-associated transcript. *Science.* 2000; 287(5457):1500–1503. [PubMed: 10688801]
31. Dasgupta G, Chentoufi Aa, Kalantari-Dehaghi M, et al. Immunodominant “Asymptomatic” Herpes Simplex Virus Type 1 and Type 2 Protein Antigens Identified by Probing Whole ORFome Microarrays By Serum Antibodies From Seropositive Asymptomatic Versus Symptomatic Individuals. *J Virology.* 2012 In press. A first genome-wide discovery of symptomatic and asymptomatic antigens from HSV-1 and HSV-2.
32. Wald A, Corey L, Cone R, Hobson A, Davis G, Zeh J. Frequent genital herpes simplex virus 2 shedding in immunocompetent women. Effect of acyclovir treatment. *J Clin Invest.* 1997; 99(5):1092–1097. [PubMed: 9062368]
33. Knaup B, Schunemann S, Wolff Mh. Subclinical reactivation of herpes simplex virus type 1 in the oral cavity. *Oral microbiology and immunology.* 2000; 15(5):281–283. [PubMed: 11154417]
34. Wald A, Zeh J, Selke S, et al. Reactivation of genital herpes simplex virus type 2 infection in asymptomatic seropositive persons. *The New England journal of medicine.* 2000; 342(12):844–850. [PubMed: 10727588]
35. Wald A, Ashley-Morrow R. Serological testing for herpes simplex virus (HSV)-1 and HSV-2 infection. *Clin Infect Dis.* 2002; 35(Suppl 2):S173–182. [PubMed: 12353203]
36. Heds. Acyclovir for the prevention of recurrent herpes simplex virus eye disease. *Herpetic Eye Disease Study Group. The New England journal of medicine.* 1998; 339:300–306. [PubMed: 9696640]
37. Leigh, Jf; Acharya, N.; Cevallos, V.; Margolis, Tp. Does asymptomatic shedding of herpes simplex virus on the ocular surface lead to false-positive diagnostic PCR results? *Br J Ophthalmol.* 2008; 92(3):435–436. [PubMed: 18314406]
38. Freeman, Ml; Sheridan, Bs; Bonneau, Rh; Hendricks, Rl. Psychological stress compromises CD8+ T cell control of latent herpes simplex virus type 1 infections. *J Immunol.* 2007; 179(1):322–328. [PubMed: 17579052]
39. Tronstein E, Johnston C, Huang Ml, et al. Genital shedding of herpes simplex virus among symptomatic and asymptomatic persons with HSV-2 infection. *JAMA.* 2011; 305(14):1441–1449. [PubMed: 21486977]

40. Kaufman, He; Azcuay, Am; Varnell, Ed; Sloop, Gd; Thompson, Hw; Hill, Jm. HSV-1 DNA in tears and saliva of normal adults. *Invest Ophthalmol Vis Sci*. 2005; 46(1):241–247. [PubMed: 15623779]
41. Liesegang, Tj. Herpes simplex virus epidemiology and ocular importance. *Cornea*. 2001; 20(1):1–13. [PubMed: 11188989]
42. Chentoufi, Aa; Binder, Nr; Berka, N., et al. Asymptomatic human CD4+ cytotoxic T-cell epitopes identified from herpes simplex virus glycoprotein B. *J Virol*. 2008; 82(23):11792–11802. Two asymptomatic and one symptomatic human CD4 epitopes identified in HSV-1 gB. [PubMed: 18799581]
43. Tigges, Ma; Koelle, D.; Hartog, K.; Sekulovich, Re; Corey, L.; Burke, Rl. Human CD8+ herpes simplex virus-specific cytotoxic T-lymphocyte clones recognize diverse virion protein antigens. *J Virol*. 1992; 66(3):1622–1634. [PubMed: 1310769]
44. Stanberry, Lr. Evaluation of herpes simplex virus vaccines in animals: the guinea pig vaginal model. *Rev Infect Dis*. 1991; 13 (Suppl 11):S920–923. [PubMed: 1664129]
45. Bernstein, Di; Stanberry, Lr. Herpes simplex virus vaccines. *Vaccine*. 1999; 17(13–14):1681–1689. [PubMed: 10194823]
46. Stanberry, Lr. Clinical trials of prophylactic and therapeutic herpes simplex virus vaccines. *Herpes*. 2004; 11 (Suppl 3):161A–169A.
47. De Bruyn G, Vargas-Cortez M, Warren T, et al. A randomized controlled trial of a replication defective (gH deletion) herpes simplex virus vaccine for the treatment of recurrent genital herpes among immunocompetent subjects. *Vaccine*. 2006; 24(7):914–920. [PubMed: 16213066]
48. Koelle, Dm. Vaccines for herpes simplex virus infections. *Curr Opin Investig Drugs*. 2006; 7(2): 136–141.
49. Da Costa, Xj; Morrison, La; Knipe, Dm. Comparison of different forms of herpes simplex replication-defective mutant viruses as vaccines in a mouse model of HSV-2 genital infection. *Virology*. 2001; 288(2):256–263. [PubMed: 11601897]
50. Geiss, Bj; Smith, Tj; Leib, Da; Morrison, La. Disruption of virion host shutoff activity improves the immunogenicity and protective capacity of a replication-incompetent herpes simplex virus type 1 vaccine strain. *J Virol*. 2000; 74(23):11137–11144. [PubMed: 11070010]
51. Johnston C, Koelle Dm, Wald A. HSV-2: in pursuit of a vaccine. *J Clin Invest*. 2011; 121(12): 4600–4609. [PubMed: 22133885]
52. Oxman, Mn. Zoster vaccine: current status and future prospects. *Clin Infect Dis*. 2010; 51(2):197–213. [PubMed: 20550454]
53. Weinberg A, Zhang Jh, Oxman Mn, et al. Varicella-zoster virus-specific immune responses to herpes zoster in elderly participants in a trial of a clinically effective zoster vaccine. *J Infect Dis*. 2009; 200(7):1068–1077. [PubMed: 19712037]
54. Da Costa X, Kramer Mf, Zhu J, Brockman Ma, Knipe Dm. Construction, phenotypic analysis, and immunogenicity of a UL5/UL29 double deletion mutant of herpes simplex virus 2. *J Virol*. 2000; 74(17):7963–7971. [PubMed: 10933704]
55. Da Costa, Xj; Jones, Ca; Knipe, Dm. Immunization against genital herpes with a vaccine virus that has defects in productive and latent infection. *Proc Natl Acad Sci U S A*. 1999; 96(12):6994–6998. [PubMed: 10359827]
56. Da Costa, Xj; Bourne, N.; Stanberry, Lr; Knipe, Dm. Construction and characterization of a replication-defective herpes simplex virus 2 ICP8 mutant strain and its use in immunization studies in a guinea pig model of genital disease. *Virology*. 1997; 232(1):1–12. [PubMed: 9185583]
57. Swetter, Sm; Hill, El; Kern, Er, et al. Chronic vulvar ulceration in an immunocompetent woman due to acyclovir-resistant, thymidine kinase-deficient herpes simplex virus. *J Infect Dis*. 1998; 177(3):543–550. [PubMed: 9498430]
58. Spector, Fc; Kern, Er; Palmer, J., et al. Evaluation of a live attenuated recombinant virus RAV 9395 as a herpes simplex virus type 2 vaccine in guinea pigs. *J Infect Dis*. 1998; 177(5):1143–1154. [PubMed: 9592996]
59. Branco, Fj; Fraser, Nw. Herpes simplex virus type 1 latency-associated transcript expression protects trigeminal ganglion neurons from apoptosis. *J Virol*. 2005; 79(14):9019–9025. [PubMed: 15994795]

60. Prichard, Mn; Kaiwar, R.; Jackman, Wt, et al. Evaluation of AD472, a live attenuated recombinant herpes simplex virus type 2 vaccine in guinea pigs. *Vaccine*. 2005; 23(46–47):5424–5431. [PubMed: 15950327]
61. Reszka, Nj; Dudek, T.; Knipe, Dm. Construction and properties of a herpes simplex virus 2 dl5–29 vaccine candidate strain encoding an HSV-1 virion host shutoff protein. *Vaccine*. 2010; 28(15): 2754–2762. [PubMed: 20117270]
62. Liu J, O'brien Kl, Lynch Dm, et al. Immune control of an SIV challenge by a T-cell-based vaccine in rhesus monkeys. *Nature*. 2009; 457(7225):87–91. [PubMed: 18997770]
63. Hoshino Y, Pesnicak L, Dowdell Kc, et al. Comparison of immunogenicity and protective efficacy of genital herpes vaccine candidates herpes simplex virus 2 dl5-29 and dl5-29-41L in mice and guinea pigs. *Vaccine*. 2008; 26(32):4034–4040. [PubMed: 18565628]
64. Dudek T, Mathews Lc, Knipe Dm. Disruption of the U(L)41 gene in the herpes simplex virus 2 dl5-29 mutant increases its immunogenicity and protective capacity in a murine model of genital herpes. *Virology*. 2008; 372(1):165–175. [PubMed: 18006033]
65. Dudek T, Knipe Dm. Replication-defective viruses as vaccines and vaccine vectors. *Virology*. 2006; 344(1):230–239. [PubMed: 16364753]
66. Hoshino Y, Qin J, Follmann D, Cohen Ji, Straus Se. The number of herpes simplex virus-infected neurons and the number of viral genome copies per neuron correlate with the latent viral load in ganglia. *Virology*. 2008; 372(1):56–63. [PubMed: 18045638]
67. Halford, Wp; Puschel, R.; Gershburg, E.; Wilber, A.; Gershburg, S.; Rakowski, B. A live-attenuated HSV-2 ICP0 virus elicits 10 to 100 times greater protection against genital herpes than a glycoprotein D subunit vaccine. *PLoS One*. 2011; 6(3):e17748. An example of a promising live-attenuated HSV-2 vaccine that induces a strong protection in animal models. [PubMed: 21412438]
68. Halford, Wp; Puschel, R.; Rakowski, B. Herpes simplex virus 2 ICP0 mutant viruses are avirulent and immunogenic: implications for a genital herpes vaccine. *PLoS One*. 2010; 5(8):e12251. [PubMed: 20808928]
69. Halford, Wp; Weisend, C.; Grace, J., et al. ICP0 antagonizes Stat 1-dependent repression of herpes simplex virus: implications for the regulation of viral latency. *Viol J*. 2006; 3:44. [PubMed: 16764725]
70. Franchini M, Hefti H, Vollstedt S, et al. Dendritic cells from mice neonatally vaccinated with modified vaccinia virus Ankara transfer resistance against herpes simplex virus type I to naive one-week-old mice. *J Immunol*. 2004; 172(10):6304–6312. [PubMed: 15128820]
71. Abril C, Engels M, Liman A, et al. Both viral and host factors contribute to neurovirulence of bovine herpesviruses 1 and 5 in interferon receptor-deficient mice. *J Virol*. 2004; 78(7):3644–3653. [PubMed: 15016885]
72. Franchini M, Abril C, Schwerdel C, Ruedl C, Ackermann M, Suter M. Protective T-cell-based immunity induced in neonatal mice by a single replicative cycle of herpes simplex virus. *J Virol*. 2001; 75(1):83–89. [PubMed: 11119576]
73. Osorio Y, Cohen J, Ghiasi H. Improved protection from primary ocular HSV-1 infection and establishment of latency using multigenic DNA vaccines. *Invest Ophthalmol Vis Sci*. 2004; 45(2): 506–514. [PubMed: 14744892]
74. Ghiasi H, Cai S, Slanina S, Nesburn Ab, Wechsler Sl. Vaccination of mice with herpes simplex virus type 1 glycoprotein D DNA produces low levels of protection against lethal HSV-1 challenge. *Antiviral Res*. 1995; 28(2):147–157. [PubMed: 8585768]
75. Pardoe I, Dargan D. PREPS and L-particles: a new approach to virus-like particle vaccines. *Expert Rev Vaccines*. 2002; 1(4):427–432. [PubMed: 12901580]
76. Muller, Wj; Dong, L.; Vilalta, A., et al. Herpes simplex virus type 2 tegument proteins contain subdominant T-cell epitopes detectable in BALB/c mice after DNA immunization and infection. *J Gen Virol*. 2009; 90(Pt 5):1153–1163. [PubMed: 19264627]
77. Muller, Wj; Orgun, Nn; Dong, L.; Koelle, Dm; Huang, Ml; Way, Ss. Recombinant *Listeria monocytogenes* expressing an immunodominant peptide fails to protect after intravaginal challenge with herpes simplex virus-2. *Arch Virol*. 2008; 153(6):1165–1169. [PubMed: 18443737]
78. Stanberry, Lr; Spruance, Sl; Cunningham, Al, et al. Glycoprotein-D-adjuvant vaccine to prevent genital herpes. *N Engl J Med*. 2002; 347(21):1652–1661. [PubMed: 12444179]

79. BenMohamed L, Belkaid Y, Loing E, Brahimi K, Gras-Masse H, Druilhe P. Systemic immune responses induced by mucosal administration of lipopeptides without adjuvant. *Eur J Immunol.* 2002; 32(8):2274–2281. [PubMed: 12209640]
80. BenMohamed L, Krishnan R, Auge C, Primus Jf, Diamond Dj. Intranasal administration of a synthetic lipopeptide without adjuvant induces systemic immune responses. *Immunology.* 2002; 106(1):113–121. [PubMed: 11972639]
81. Bourne N, Bravo Fj, Francotte M, et al. Herpes simplex virus (HSV) type 2 glycoprotein D subunit vaccines and protection against genital HSV-1 or HSV-2 disease in guinea pigs. *J Infect Dis.* 2003; 187(4):542–549. [PubMed: 12599070]
82. Zhang X, Chentoufi Aa, Dasgupta G, et al. A genital tract peptide epitope vaccine targeting TLR-2 efficiently induces local and systemic CD8+ T cells and protects against herpes simplex virus type 2 challenge. *Mucosal Immunol.* 2009; 2(2):129–143. [PubMed: 19129756]
83. Corey L, Langenberg Ag, Ashley R, et al. Recombinant glycoprotein vaccine for the prevention of genital HSV-2 infection: two randomized controlled trials. Chiron HSV Vaccine Study Group. *Jama.* 1999; 282(4):331–340. [PubMed: 10432030]
84. Hartley C. Immunologic strategies for herpes vaccination. *Jama.* 2000; 283(6):746. author reply 746–747. [PubMed: 10683052]
85. Friedman, Hm. Immunologic strategies for herpes vaccination. *Jama.* 2000; 283(6):746. author reply 746–747. [PubMed: 10683051]
86. Chentoufi, Aa; BenMohamed, L. Future Viral Vectors for the Delivery of Asymptomatic Herpes Epitopes-Based Immunotherapeutic Vaccines. *Future Virology.* 2010; 5:1–4.
87. Dasgupta G, Nesburn Ab, Wechsler Sl, Benmohamed L. Developing an asymptomatic mucosal herpes vaccine: the present and the future. *Future Microbiol.* 2010; 5:1–4. [PubMed: 20020824]
88. Rajcani J, Durmanova V. Developments in herpes simplex virus vaccines: old problems and new challenges. *Folia Microbiol (Praha).* 2006; 51(2):67–85. [PubMed: 16821715]
89. Eriksson K, Bellner L, Gorander S, et al. CD4(+) T-cell responses to herpes simplex virus type 2 (HSV-2) glycoprotein G are type specific and differ in symptomatic and asymptomatic HSV-2-infected individuals. *J Gen Virol.* 2004; 85(Pt 8):2139–2147. [PubMed: 15269352]
90. Chentoufi, Aa; Zhang, X.; Lamberth, K., et al. HLA-A*0201-restricted CD8+ cytotoxic T lymphocyte epitopes identified from herpes simplex virus glycoprotein D. *J Immunol.* 2008; 180(1):426–437. First asymptomatic CD8 T cell epitopes identified in HSV-1 gD. [PubMed: 18097044]
91. Zhang X, Castelli Fa, Zhu X, Wu M, Maillere B, BenMohamed L. Gender-dependent HLA-DR-restricted epitopes identified from herpes simplex virus type 1 glycoprotein D. *Clin Vaccine Immunol.* 2008; 15(9):1436–1449. [PubMed: 18667634]
92. Kim, Sk; Cornberg, M.; Wang, Xz; Chen, Hd; Selin, Lk; Welsh, Rm. Private specificities of CD8 T cell responses control patterns of heterologous immunity. *J Exp Med.* 2005; 201(4):523–533. [PubMed: 15710651]
93. Welsh, Rm; Selin, Lk. No one is naive: the significance of heterologous T-cell immunity. *Nat Rev Immunol.* 2002; 2(6):417–426. [PubMed: 12093008]

Executive Summary

- Herpes simplex viruses type 1 and type 2 (HSV-1 & HSV-2) are infectious pathogens that cause serious diseases at every stage of life including fatal disseminated disease in newborns, to cold sores, genital ulcerations, eye disease, and fatal encephalitis in adults
- Considering the limited success of the recent herpes clinical vaccine trial [1], new vaccine strategies are needed.
- The immune mechanism(s) by which asymptomatic patients control herpetic disease and symptomatic patients do not remains to be fully elucidated [42]. Identifying these mechanisms, or at least the viral antigens (Ags) and epitopes involved, is critical to understanding how to protect against recurrent herpetic disease and for rational advances in therapeutic vaccine development.
- Our recent findings show that T cells from symptomatic and asymptomatic men and women (i.e. those with and without recurrences, respectively) recognize different herpes epitopes.
- This finding breaks new ground and opens new doors to assess a new vaccine strategy: mucosal immunization with HSV-1 & HSV-2 epitopes that induce strong in vitro CD4 and CD8 T cell responses from PBMC derived from asymptomatic men and women (designated here as “asymptomatic” protective epitopes”) could boost local and systemic “natural” protective immunity, induced by wild-type infection.
- An important lesson learned from the pre-clinical and clinical vaccine trials described above is the true feasibility (i.e. practicability) of a herpes vaccine. “Common denominators” between most of the above vaccine strategies are that: (i) the cellular immunity appears to be crucial, rather than the humoral immunity, in protection against herpes; (ii) the delivered antigens contains both “symptomatic” and “asymptomatic” T cell epitopes.
- A good starting point for the development of an efficient therapeutic herpes vaccine would be to identify the matrices of protective “asymptomatic” Ags and epitopes strongly recognized by T cells from asymptomatic individuals (as illustrated in Fig. 1).
- Our study suggests that HSV infected HLA transgenic rabbits that developed recurrent corneal disease (i.e., a “symptomatic” HLA transgenic rabbit) are able to respond to asymptomatic epitope vaccination. This suggests that symptomatic individuals will be able to respond appropriately to a therapeutic asymptomatic epitope based vaccine and develop a CD8⁺ T-cell response specific to the asymptomatic epitopes in the vaccine.

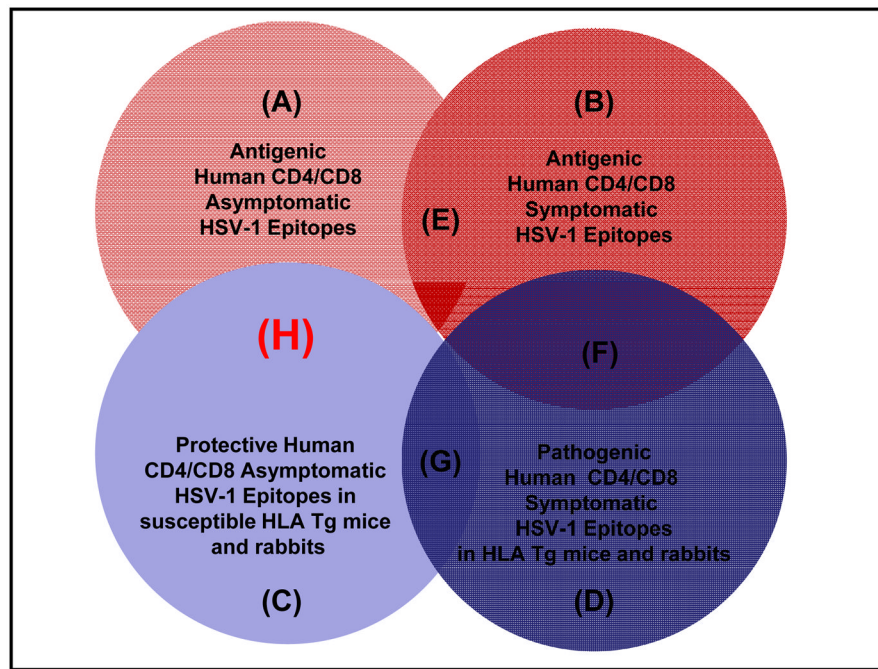


Fig. 1. Identification of “protective” asymptomatic HSV-1 T cell epitopes from HSV-1 and HSV-2

(A) “Asymptomatic” T cell epitopes that induce strong in vitro CD4 and CD8 T cell responses from PBMC derived from asymptomatic individuals are selected. (B) “Symptomatic” T cell epitopes that induce strong in vitro CD4 and CD8 T cell responses from PBMC derived from symptomatic individuals are excluded. (C) “Asymptomatic” T cell epitopes that are protective in HLA transgenic mice and rabbits are selected. (D) “Symptomatic” T cell epitopes that are pathogenic in HLA transgenic mice and rabbits are excluded. (E) A few overlapping epitopes may be strongly recognized by T cells from both symptomatic and asymptomatic individuals and are also excluded. (F) Only some epitopes recognized by T cells from symptomatic individuals are immunogenic in vivo in animal are also excluded. (G) Although some epitopes are immunogenic in vivo in animal models they are neither protective nor pathogenic and are therefore also excluded. The successful combination of the above criteria will determine the final asymptomatic epitopes (H) that will be included in a future multivalent “asymptomatic” vaccine against ocular herpes. Note that the selected asymptomatic epitopes also induce a weak T cell response in symptomatic individuals. Thus a repertoire of “asymptomatic” T cells do exist in the symptomatic individuals do exist but it must to be boosted by the future “asymptomatic therapeutic vaccine in order to induce protection.