Vaccine xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Current status and prospects for development of an HSV vaccine☆

Christine Johnston[a,b,*], David M. Koelle[a,b,c,e,f], Anna Wald[a,b,c,d]

[a] Department of Medicine, University of Washington, Seattle, WA, United States
[b] Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA, United States
[c] Department of Laboratory Medicine, University of Washington, Seattle, WA, United States
[d] Department of Epidemiology, University of Washington, Seattle, WA, United States
[e] Department of Global Health, University of Washington, Seattle, WA, United States
[f] Benaroya Research Institute, Seattle, WA, United States

ARTICLE INFO

Article history:
Received 25 March 2013
Received in revised form 5 June 2013
Accepted 17 August 2013
Available online xxx

Keywords:
Herpes simplex virus
Vaccine
Genital herpes

ABSTRACT

Herpes simplex virus type 2 (HSV-2) infects 530 million people, is the leading cause of genital ulcer disease, and increases the risk of HIV-1 acquisition. Although several candidate vaccines have been promising in animal models, prophylactic and therapeutic vaccines have not been effective in clinical trials thus far. Null results from the most recent prophylactic glycoprotein D2 subunit vaccine trial suggest that we must reevaluate our approach to HSV-2 vaccine development. We discuss HSV-2 pathogenesis, immunity, and vaccine efforts to date, as well as the current pipeline of candidate vaccines and design of trials to evaluate new vaccine constructs.

1. HSV epidemiology: global burden of disease

Herpes simplex virus type 2 (HSV-2) is an incurable sexually transmitted pathogen that infects over 500 million people worldwide and causes an estimated 23 million new infections annually [1]. In the United States, direct annual medical costs associated with HSV-2 are estimated to be $541 million, making it the third most costly STI after HIV-1 and human papillomavirus (HPV) [2]. HSV-2 seroprevalence ranges from 16% among 14–49 year olds in the United States [3], to >80% in areas of sub-Saharan Africa [4]. HSV-2 infection rates in heavily exposed populations are nearly 100%, suggesting universal susceptibility [5]. Seroprevalence in women is up to twice as high as men, and increases with age [3,6]. Although HSV-2 is the leading cause of genital ulcer disease (GUD) worldwide [7,8], most people are unaware of having the infection [9]. HSV-2 transmission occurs through genital-genital contact during sexual activity. HSV-2 may be transmitted in the absence of signs or symptoms of infection in the infected partner, during episodes of subclinical shedding [10]. In addition, most people who acquire HSV-2 are asymptomatic at the time of acquisition [11]. Transmission of HSV from mother to infant during birth is a serious complication of genital herpes, and can result in long-term neurologic sequelae or mortality [12]. Women who acquire HSV during pregnancy are at the highest risk of transmitting the infection [13]. With an estimated incidence of 4–31/100,000 live births [14,15], neonatal herpes is too rare to be used as an endpoint in a clinical trial. However, prevention of HSV acquisition during pregnancy is an important goal of developing an effective HSV vaccine.

The greatest public health impact of HSV-2 infection is its role in promulgating the HIV-1 epidemic. Persons with HSV-2 infection are 3-fold more likely to acquire HIV-1 infection [16]; this risk increases up to 8-fold if the exposure occurs soon after acquiring HSV-2 infection [17,18]. In HIV-1 infected persons, HIV-1 is found in HSV-2 genital ulcers [19], and persons with genital ulcers are at increased risk of transmitting HIV-1 [20]. In regions with high HSV-2 seroprevalence (>80%), 25–50% of HIV-1 infections are attributable to HSV-2 [21]. Mathematical models suggest that even moderately effective prophylactic HSV-2 vaccines would lead to a marked decrease in HIV-1 incidence if given at high coverage [22]. The biologic basis for this predisposition is the persistent mucosal inflammatory response induced by HSV-2. Genital biopsy studies have revealed that HSV-2 ulcers are associated with an infiltrate of CD4+ T-cells bearing the HIV-1 co-receptors CCR5 or CXCR4, which persists during daily antiviral therapy for HSV [23]. Histopathologic studies of foreskins from HIV-1-seronegative men demonstrate that HSV-2 seropositive men have increased concentration of CD4+ and CD8+ T-cells as compared to HSV-2 seronegative men [24].

☆ *Funding*: National Institutes of Health K24 AI071113 (AW), PO1 AI30731 (CJ, AW, DMK), R01 AI094019 (DMK).

* Corresponding author at: University of Washington Virology Research Clinic, Box 359928, 325 9th Avenue, Seattle, WA 98104, United States. Tel.: +1 206 520 4340; fax: +1 206 520 4371.
E-mail address: cjohnsto@u.washington.edu (C. Johnston).

0264-410X/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.vaccine.2013.08.066

Similar findings have been found in cervical cytobrush samples from HIV-1 negative, HSV-2 seropositive women [25].

Currently available HSV-2 prevention strategies are inadequate; each reduces the risk of transmission by approximately 50%. Evidence-based methods include use of suppressive antiviral therapy [26], disclosure of serostatus to susceptible partners [27], and consistent condom use [28]. While male circumcision decreases the risk of HSV-2 acquisition by nearly 30% [29], there are conflicting data about the role of circumcision in transmission to women [30,31]. These partly effective strategies may be useful for management of individual patients, but they are unlikely to be of public health benefit. Indeed, even with availability of suppressive antiherpes viral therapy in the USA, seroprevalence rates are similar to the pre-antiviral era [6].

2. Changing epidemiology of genital herpes: role of HSV-1

Over the past 2 decades, incident genital herpes in developed countries is increasingly caused by HSV type 1 (HSV-1), especially in persons <25 years of age [32]. This is likely due to declining seroprevalence of HSV-1 in adolescents [6], resulting in the first mucosal exposure to HSV-1 at initiation of sexual activity. As HSV-1 and HSV-2 have similar pathogenesis and host interactions, concepts for effective vaccine development may be relevant to both viruses. Infection with HSV-2 provides partial protection against HSV-1 [15], but the reverse is not true [33]. We need more information about HSV-1 genital infection, the risk of transmission to sex partners and neonates, and interactions between HIV-1 and HSV-1. Vaccines which provide protection against genital HSV-1 infection will be important to reduce the prevalence of genital herpes and its' sequelae.

3. New insights into HSV pathogenesis: frequent and dynamic reactivation

During primary infection, HSV infects epithelial cells at skin and mucosa surfaces and is transported along nerve axons to the dorsal root ganglia (DRG), where latency is established [34]. Neuronal cells are not destroyed during initial HSV infection and provide a reservoir for latent virus [35]. During reactivation the virus travels from the ganglia back to the skin and results in detection of virus ("viral shedding") from epithelial surfaces. Viral reactivation is most often asymptomatic, but may be associated with genital symptoms or ulcers. Recent studies have demonstrated that episodes of genital HSV reactivation last a median of 13 h and are likely rapidly cleared by host responses [36–38]. These may include tissue resident memory (T_{RM}) T cells, discussed below, and suggest that frequent antigen exposure stimulates a chronic immune response in the mucosa.

4. The immune response to HSV-2

Murine HSV models are useful for basic HSV immunology [39], but mimic neither primary nor recurrent human infection. Guinea pigs experience recurrent infection [40], but tools for mechanistic studies are poor, and other models have practical problems or poor evidence for seroconversion [41,42]. The host and viral determinants of the heterogeneous clinical and virological manifestations of genital HSV-2 in humans are poorly understood. Identification of the components of the host immune system that contain viral reactivation from neurons and promote viral clearance from the mucosa will be essential for development of a successful HSV-2 vaccine. This information will be gained by detailed immunologic and genetic studies of persons with well-defined HSV-2 severity.

The importance of the innate immune system has been demonstrated by observations that human mutations in a TLR3-centric pathway are associated with severe primary HSV infection [43]. While TLR3 is required for priming CD8+ T-cell responses to HSV infection [44] and can be manipulated by adjuvants [45], data linking variation in TLR3 and recurrent HSV severity in humans are conflicting [46–48]. Variation in other host loci involved in immunity may be associated with HSV severity [49], but the ability manipulate these with vaccines is limited at this time. These findings suggest that adjuvant which promotes innate immune responses may be important for an HSV vaccine.

Antibody-driven vaccines remain of intense interest. The rationale for pursuing neutralizing antibodies is based on the biology of perinatal HSV transmission in the absence *vs.* presence of pre-existing maternal antibody [15], and animal passive transfer studies [50]. Neutralizing antibody titers correlate with protection to HPV infection, another epithelial STI [51]. The step-wise process of HSV entry, starting with glycoprotein (g)D binding to cell-type specific high affinity receptors and subsequent gB-mediated fusion with mandatory involvement by the gH-gL heterodimer, is becoming clear from structural biology and mutational work [52–55]. Recent advances in human B-cell cloning, high throughput antibody screening, sequencing and expression, and crystallization of complexes of antigens with highly favorable antibodies, as exemplified by HIV-1 and influenza [56,57] could yield improved HSV immunogen designs.

Evidence is now emerging in both human and murine studies that local T-cells can serve as epithelial sentinels to provide a surveillance function to modulate primary and re-infection episodes. Using *in situ* methods, prolonged residence of HSV-2-specific CD8+ T-cells was documented at the dermo-epidermal junction (DEJ) in humans [58]. These cells have an activated phenotype and a unique expression pattern of homing-related molecules [59]. Elegant murine studies prove prolonged residence of HSV-specific CD8+ T-cells that is spatially limited to sites of previous infection and capable of mediating local protection to exogenous re-scarification, the best model of recurrence in this system [60]. Recently, systemic vaccination with replication-competent, attenuated HSV-2 was followed by a chemoattractant therapy given vaginally in mice [39]. This was found to "pull" vaccine-primed cells to the site of challenge, and to mediate long-lived functional protection [39], providing direct evidence of the importance of CD8 T cells. While vaginal administration of pro-inflammatory chemokines or upstream innate stimuli is challenging in humans, this is an important conceptual advance, establishing the ability to develop tissue resident-memory (T_{RM}) cells without local infection. Mathematical models suggest that small fluctuations in T_{RM} levels could tip the balance between subclinical and clinical reactivation [38]. Therefore, understanding protective T cell responses and stimulating such responses through a vaccine is an ongoing research priority.

At the whole pathogen level, the integrated CD4 and CD8 response in chronically infected persons occupies about 0.1 to 3% of the PBMC compartment [61,62]. We found no correlation between the magnitude or functionality (IFN-γ, IL-2, TNF-α) of the integrated CD4 responses to whole HSV-2 and shedding or clinical severity in a cross-section survey [61]. Thus, target CD4 levels for preventative vaccines are hard to define, and simply boosting pre-existing CD4 responses may not be rational for immunotherapy. Because HSV-1 and HSV-2 have immune evasive mechanisms and are directly cytotoxic to activated lymphocytes, measuring the size or phenotype of the integrated CD8 response to the whole virus has been challenging. Whether a critical level or phenotype of circulating CD8 responses will correlate with vaccine success is unknown.

Recently developed tools which contain every HSV-1 and HSV-2 open reading frame allow examination of responses at antigen- and epitope-specific levels [62,63]. Using this unbiased proteomic approach, we found that CD4+ and CD8+ T-cells in HSV-1 infected humans recognize an average of 17 and 22 ORFs, respectively, with

Please cite this article in press as: Johnston C, et al. Current status and prospects for development of an HSV vaccine. Vaccine (2013), http://dx.doi.org/10.1016/j.vaccine.2013.08.066