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Identification of novel virus-specific antigens by CD4$^+$ and CD8$^+$ T cells from asymptomatic HSV-2 seropositive and seronegative donors

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ABSTRACT

Reactivation of latent herpes simplex virus 2 (HSV-2) infections can be characterized by episodic recurrent genital lesions and/or viral shedding. We hypothesize that infected (HSV-2^{pos}) asymptomatic individuals have acquired T cell responses to specific HSV-2 antigen(s) that may be an important factor in controlling their recurrent disease symptoms. Our proteomic screening technology, ATLAS™, was used to characterize the antigenic repertoire of T cell responses in infected (HSV-2^{pos}) and virus-exposed seronegative (HSV-2^{neg}) subjects. T cell responses, determined by IFN-γ secretion, were generated to gL, UL2, UL11, UL21, ICP4, ICP0, ICP47 and UL40 with greater magnitude and/or frequency among cohorts of exposed HSV-2^{neg} or asymptomatic HSV-2^{pos} individuals, compared to symptomatic recurrent HSV-2^{pos} subjects. T cell antigens recognized preferentially among individuals who are resistant to infection or who are infected and have mild or no clinical disease may provide new targets for the design of vaccines aimed at treating and/or preventing HSV-2 infection.

Introduction

Genital infection with HSV is characterized by the development of painful ulcers that spontaneously resolve. The virus remains in the dorsal root ganglia of the sacral nerves but re-emerges causing clinical recurrences at variable intervals and with variable severity. With over 500 million existing infections, and 20 million new cases per year, herpes simplex virus type 2 (HSV-2) is a worldwide pandemic (Looker and Garnett, 2005). Until an effective vaccine is developed, those numbers are expected to grow. Considerable time and resources have been devoted to developing vaccines for the immunotherapy or prophylaxis of HSV-2. All vaccines tested to date have failed to meet pre-defined endpoints in clinical trials.

Studies conducted in mice suggest that control of HSV-2 will require coordinated immune responses, including both antibody production and activation of CD4$^+$ and CD8$^+$ T cells (Morrison et al., 2001; Thebeau and Morrison, 2003). A clinical trial of a vaccine targeting the HSV-2 surface glycoproteins D and B (gD2 and gB2) and adjuvanted with MF59 successfully activated CD4$^+$ T cells and neutralizing antibody production (Corey, 1999). Nevertheless, vaccination failed to prevent acquisition of HSV-2 infection, and had no impact on clinical symptoms following primary infection or on subsequent frequency of reactivation. gD and gB have been shown to be important targets of neutralizing antibody responses to infection but their role as protective T cell antigens in humans has not been established. The latest HSV-2 clinical trial with its focus on the subgroup of double seronegative women, showed that the gD2 subunit vaccine adjuvanted with aluminum hydroxide and 3-O-deacylated monophosphoryl lipid A was effective in preventing HSV-1 genital disease or infection but not in preventing HSV-2 disease or infection (Belshe et al., 2012). Recently, Belshe et al. demonstrated a statistically significant correlation between gD antibody concentration and efficacy (Belshe et al., 2013). These clinical trial outcomes, along with findings from animal disease models (Morrison et al., 2001; Thebeau and Morrison, 2003) suggest a critical role for antibody and T cells with diverse specificities in controlling HSV-2 infection, and that incorporating antigens capable of activating CD8$^+$ T cell responses in addition to CD4$^+$ T cell responses and antibody will be essential for eliciting a protective immune response. In addition, it will be necessary to deliver these antigens with an adjuvant or delivery system that stimulates appropriate humoral and cellular immune responses.

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The clinical course of HSV-2-induced genital disease is varied. While the majority of seropositive individuals remain asymptomatic, others experience frequent recurrent outbreaks. Clearance of HSV-2 from genital herpes lesions has been associated with an influx of HSV-specific $CD8^+$ T cells, and it has been proposed that these cells play a role in immune control of symptomatic HSV-2 reactivation in genital skin (Koelle et al., 2001, 1998; Zhu et al., 2007). Several recent studies have demonstrated that T cells isolated from asymptomatic versus symptomatic subjects exhibit differential epitope specificity, implying that antigens that preferentially activate T cells of asymptomatic HSV-2 carriers may play a crucial role in controlling disease (Chentoufi et al., 2008; Dasgupta et al., 2012). In this study we use full-length or large protein fragments rather than peptides to screen T cells for potential differences between asymptomatic and symptomatic cohorts; as a result, differences at the epitope level may be missed. Recently, Johnston et al. (2014) used a HSV-2 (ORF)eome expression library to test for proliferative $CD4^+$ T cell responses in genital biopsy specimens of HSV-2 seropositive subjects. However, the focus of this study is the characterization of both $CD4^+$ and $CD8^+$ peripheral T cell antigen repertoires in HSV-2 seropositive and seronegative cohorts across a broad population, rather than a search for more restricted differences at an epitope level or the dissection of antigen-specific T cell responses at mucosal surfaces. Interestingly, Posavad et al. demonstrated that some individuals who are HSV seronegative elicited peripheral HSV-specific T cell responses despite the absence of infection, suggesting that virus-specific T cells in these individuals have been stimulated by exposure and provide protection (Posavad et al., 2010). The rational design of a vaccine that mimics this response could potentially control disease.

Identification of antigens, especially those to which $CD8^+$ T cells can respond, has been complicated by the multiple restrictions of epitope presentation in genetically diverse individuals and the requirement that proteins are processed through the cytosolic route of antigen presentation. Recent approaches to identify $CD8^+$ T cell antigens include *in silico* epitope prediction (Moutaftsi et al., 2006), generation of overlapping peptides targeting a portion of a pathogen's proteome (Braun et al., 2006; Hosken et al., 2006), and screens of random genomic libraries expressed by mammalian cells (Koelle, 2003; Starnbach et al., 2003). One major limitation of these approaches is the failure to identify antigens capable of being presented by the numerous HLA class I haplotypes representing a diverse human population. In addition, these screens do not guarantee comprehensive coverage of the entire pathogen proteome, excluding potentially relevant antigens. Indeed, the high cost of synthesizing numerous overlapping peptides generally limits the possibility of including the entire proteome. The microbial genomic approach does have the potential to provide complete coverage of the genome by translation of non-annotated ORFs and out-of-frame products. In contrast, our library platform is built using annotated ORFs and therefore does not necessarily include undocumentated ORFs, although it is possible that transcriptional variants are generated within the *E. coli* expression constructs.

The HSV-2 genome encodes 74 unique open reading frames (Dolan et al., 1998), accounting for potentially hundreds of T cell epitopes. We hypothesize that identification of HSV-2 proteins that correlate with clinical evidence of protection against disease in individuals who have been naturally exposed to virus, greatly enhances the odds of developing a successful vaccine candidate by significantly narrowing the field of potential antigens. To uncover these specific T cell antigens from a large pool of protein candidates, we created a high throughput, proteomic technology that facilitates unbiased and comprehensive identification of both $CD4^+$ and $CD8^+$ T cell antigens. This antigen screening system has been used previously to identify $CD4^+$ T cell antigens from *S. pneumoniae* (Li et al., 2012; Moffitt et al., 2011), and $CD4^+$ and $CD8^+$ T cell antigens from *C. trachomatis* (Picard et al., 2012; Roan et al., 2006) and *P. falciparum* (unpublished data). This method consists of two components: (1) bacterial libraries, expressing cLLO (cytoplasmic variant of listeriolysin O) for $CD8^+$ T cell screening and without cLLO expression for $CD4^+$ T cell screening, comprising all proteins predicted to be expressed by the pathogen; and (2) an *in vitro* immunological screening system that allows discernment of which antigens are capable of inducing the most potent T cell responses in human cells.

In this study, we built a complete HSV-2 proteomic library and screened it with $CD8^+$ and $CD4^+$ T cells from a total of 147 HSV-2-infected and uninfected individuals to identify viral antigens that elicited IFN-γ secretion. Nomination of viral antigens for further development as vaccine candidates is based on difference in responses to antigens among individuals who control the disease or resist infection ("protected") and those who do not ("unprotected"). The data were analyzed with three main foci: 1) determination of the overall frequency with which individuals respond to HSV-2-derived antigens; 2) evaluation of differences in frequency of responses between "protected" and "unprotected" individuals; and 3) evaluation of differences in the magnitude of responses between cohorts. Analysis of human cohorts displaying a spectrum of clinical phenotypes to HSV-2 infection has identified protein antigens able to induce T cells targeting HSV-2 which correlate with control of infection and/or disease.

Results

Clinical and demographic characteristics of cohorts

Of the 147 subjects recruited into the study, 92 were HSV-2^{pos} and 55 were HSV-2^{neg}. The HSV-2^{pos} subjects were categorized according to recurrence frequency, and distributed into two main cohorts: 52 who were symptomatic, with a median of five recurrences per year, which were defined as "unprotected"; and 40 who had a documented primary outbreak but reported no known recurrences since, who were characterized as asymptomatic, and defined as "protected" (Table 1). The recurrers could be further broken out into those who had frequent (four or more) and those with infrequent recurrences (less than four) annually. The symptomatic cohort had a median age of 38 years, with 71% female; the asymptomatic cohort had a median age of 34 years, with 61% female. Fifty-five subjects were HSV-2^{neg}, of whom 37 were characterized as "exposed", due to an ongoing sexual relationship with HSV-2^{pos} partners and who were also defined as "protected". Eighteen HSV-2^{neg} subjects had no known exposure to HSV-2, thus were defined as "unexposed". The median age of the exposed cohort was 38 years, with 46% female, while that of the unexposed cohort was 29 years of age, with 67% female. As part of the recruitment criteria, none of the HSV-2^{pos} subjects were seropositive for HSV-1 (HSV-1^{pos}); however 32 of 55 HSV-2^{neg} subjects were HSV-1^{pos} (23 HSV-2 exposed and 9 unexposed).

To rule out the possibility that one HLA class I supertype was overrepresented in respective "protected" (i.e. exposed seronegative) and "unprotected" (i.e. HSV-2^{pos} symptomatic) cohorts (Supplementary Table 2), we examined the distribution of the nine defined HLA class I supertypes among subjects representing each group (Sette and Sidney, 1999; Sidney et al., 2008). Eight of the nine defined supertypes were present in the population sent for typing; one supertype, HLA-B62, was absent. As shown in Supplementary Table 2, chi-squared tests comparing 19 HSV-2^{neg} exposed, 20 HSV-2^{pos} protected (asymptomatic) subjects and 31 HSV-2^{pos} unprotected (symptomatic) individuals showed no