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Vaccination with a HSV-2 UL24 mutant induces a protective immune response in murine and guinea pig vaginal infection models

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ABSTRACT

The rational design and development of genetically attenuated HSV-2 mutant viruses represent an attractive approach for developing both prophylactic and therapeutic vaccines for genital herpes. Previously, HSV-2 UL24 was shown to be a virulence determinant in both murine and guinea pig vaginal infection models. An UL24-βgluc insertion mutant produced syncytial plaques and replicated to nearly wild type levels in tissue culture, but induced little or no pathological effects in recipient mice or guinea pigs following vaginal infection. Here we report that immunization of mice or guinea pigs with high or low doses of UL24-βgluc elicited a highly protective immune response. UL24-βgluc immunization *via* the vaginal or intramuscular routes was demonstrated to protect mice from a lethal vaginal challenge with wild type HSV-2. Moreover, antigen re-stimulated splenic lymphocytes harvested from immunized mice exhibited both HSV-2 specific CTL activity and IFN-γ expression. Humoral anti-HSV-2 responses in serum were Th1-polarized ($IgG_{2a} > IgG_1$) and contained high-titer anti-HSV-2 neutralizing activity. Guinea pigs vaccinated subcutaneously with UL24-βgluc or the more virulent parental strain (186) were challenged with a heterologous HSV-2 strain (MS). Acute disease scores were nearly indistinguishable in guinea pigs immunized with either virus. Recurrent disease scores were reduced in UL24-βgluc immunized animals but not to the same extent as those immunized with strain 186. In addition, challenge virus was not detected in 75% of guinea pigs subcutaneously immunized with UL24-βgluc. In conclusion, disruption of the UL24 gene is a prime target for the development of a genetically attenuated live HSV-2 vaccine.

1. Introduction

Despite years of study, the need exists for an effective HSV-2 vaccine. This was underscored in 2010 at the CDC's National STD Conference where it was reported that the prevalence of HSV-2 in the United States remains high at 16.2% (http://www.cdc.gov/std/Herpes/herpes-NHANES-2010.htm [1]).

While there is still no approved HSV-2 vaccine for either prophylactic or therapeutic treatment, a number of experimental approaches have been pursued [2,3] including subunit vaccines with or without cytokines, DNA vaccines with or without cytokines, replication competent and defective viral vectored vaccines, and rationally designed attenuated vaccines [4–26]. Each approach has its own advantages and disadvantages with regards to safety, immunogenicity, and efficacy [27].

HSV-2 subunit vaccines are advantageous with respect to safety but by themselves, typically induce a Th2-like immune response, which may not be optimal for prophylactic or therapeutic protection [6,28]. Cooper et al. showed that a gD subunit vaccine could be shifted toward a Th1-like response when delivered with a biologically active adjuvant such as IL-12 [6]. However, adjuvants such as IL-12 have safety considerations associated with clinical use [29]. Recently, a large phase 3 clinical efficacy study with the goal of inducing neutralizing antibodies by vaccination with adjuvant and glycoprotein D was completed [30]. Although the vaccine showed some efficacy in preventing HSV-1 genital disease and infection, it was not effective in preventing HSV-2 disease or infection. These disappointing results suggest that neutralizing antibody alone may not be sufficient for protection and support the development of vaccines that can induce both neutralizing antibody and robust Th1 polarized T cell responses. Morrison showed that a $CD4^+$ CTL response is critical for protection against HSV-2 [31]. Furthermore, induction of a Th1 polarized response is likely necessary in therapeutic indications of genital herpes where T cell control of reactivation is important [2,32,33].

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Recombinant viral vectored vaccines have been demonstrated to induce strong specific Th1 responses [4,19,20,34–36]. This approach also has safety considerations that vary based on the vector. Furthermore, pre-existing anti-vector immunity and/or the induction of anti-vector immunity following immunization are potential problems shared by all vectored vaccines. For example, anti-vector immunity could diminish or even prevent vaccine antigen expression and thereby reduce efficacy.

Replication competent vaccines express multiple antigenic gene products and induce more robust immune responses compared to non-replicating vaccines [37]. In addition, efficacy may be enhanced by the induction of a strong Th1 response [38]. The challenge with attenuated vaccines is retaining immunogenicity without compromising host safety; particularly with neurovirulent viruses that establish latent infections. Hence, it is preferable to develop a vaccine platform where genes encoding neurovirulence factors are at a minimum altered, and wherever possible, deleted [39–48].

Deletion of the HSV-2 UL24 gene has not been investigated as a possible attenuated vaccine platform. Both HSV-1 and HSV-2 UL24 mutants display a syncytial plaque morphology and hence, on the surface, the *in vitro* phenotype suggests that the UL24 gene product is involved in mediating fusion events during infection and/or spread [49–51]. Pearson et al. showed that the UL24 gene product promotes nuclear egress of nucleocapsids during HSV-1 infection, possibly though effects on nucleoli including the redistribution of nucleolin [52]. Previously, we described a UL24-βgluc mutant virus (UL24-βgluc) containing an intragenic insertion with significantly reduced pathogenicity in both guinea pig and murine infection models when compared to the parental HSV-2 strain [53]. Along with these findings, there are three additional aspects that suggest that UL24 mutation might be useful in attenuated HSV vaccine design: (1) UL24-βgluc displays a significant syncytial plaque phenotype that may evoke a vigorous innate immune response including production of type I interferons [54], (2) equine herpes virus 1 (EHV-1) ORF37 (the UL24 homolog) was shown to be a neuropathogenicity determinate in a mouse encephalitis model [55], and (3) HSV-1 UL24 mutants replicate in cell culture and to some extent at the initial site of inoculation but are limited in spread to the nervous system – *i.e.* UL24 is a determinant of neuroinvasiveness [56].

In this study, mice immunized with a HSV-2 UL24 mutant (UL24-βgluc) *via* the vaginal, footpad or intramuscular route, were protected from lethal vaginal challenge with 100 50% lethal doses (LD_{50s}) of wild type HSV-2. All routes of administration elicited robust humoral and cellular responses to HSV-2 antigens. The T helper response was Th1 oriented as demonstrated by strong IFN-γ expression following antigenic *in vitro* re-stimulation. Protection was also observed after subcutaneous immunization of guinea pigs and challenge virus was not detected in 75% of guinea pigs subcutaneously immunized with UL24-βgluc. These results suggest that UL24-βgluc is a viable starting point for the development of an attenuated HSV-2 prophylactic vaccine and that other candidate vaccines may benefit from the incorporation of the UL24 mutation as part of their platform.

2. Materials and methods

2.1. Viruses and cells

Isolation and characterization of the UL24-βgluc mutant were described previously [53]. UL24-βgluc, HSV-2 (strain 186) and HSV-2 (strain MS) were grown and titered on Vero cell monolayers (ATCC, Manassas, VA).

2.2. Immunization and sample collection

Eight-week-old female BALB/c mice were obtained from Taconic Laboratories (Germantown, New York). All animal care and procedures conformed to Institutional Animal Care and Use Committee guidelines. Transponders (BioMedic Data Systems Inc., Rockville, MD) were inserted subcutaneously andused to identify mice and to aid the recording body weights and temperatures.

Five days prior to intravaginal immunization, mice received 2.0 mg DEPO-PROVERA® (Pharmacia & Upjohn Company, Kalamazoo, MI) subcutaneously in order to increase their susceptibility to HSV-2 vaginal infection [57]. Prior to inoculation, mice were anesthetized and their vaginas swabbed with a sterile PBS-soaked Dacron polyester tip applicator (Puritan, Guilford, ME) to remove vaginal mucous. Varying doses of virus were instilled into the vaginal vault using a micropipettor (0.01 ml/dose).

For intramuscular or footpad routes of administration, 1.25×10^4 PFU of UL24-βgluc was injected into the calf muscle or footpad respectively.

Four or eight weeks after immunization, serum samples and spleens were harvested from mice to assess the immune response.

2.3. Mouse vaginal challenge model

Five days prior to intravaginal challenge, mice were treated with DEPO-PROVERA® as indicated above. Immunized and age-matched naive littermates received a dose of 2.5×10^4 (100 LD_{50s}) of wild-type HSV-2 strain 186. Virus samples were collected from the vagina three days post challenge using Dacron swabs that were subsequently dipped into 1 ml DMEM culture medium and frozen at −70 °C. Mice were monitored daily for 1 month for mortality and disease severity using the following scale: (0) no symptoms, (1) vaginal erythema, (2) vaginal erythema and edema, (3) vaginal herpetic lesions, (4) unilateral paralysis or severe genital ulceration with hair loss from genital and surrounding tissue, and (5) bilateral paralysis or death. Surviving mice and a second set of age-matched naive mice were re-challenged 6 months after the first challenge with 100 LD_{50s} of wild-type HSV-2. Mice achieving a score of 5 or having a body temperature drop to 34 °C were immediately euthanized.

2.4. HSV-2 ELISA

Individual sera (10 per group) were evaluated for HSV-2 specific antibody responses by employing a standard ELISA protocol using microtiter plates coated with purified HSV-2 lysate (Advanced Biotechnologies, Columbia, MD) [58]. The endpoint titer was defined as the reciprocal of the greatest serum dilution resulting in an $OD_{405\,nm}$ that was equal to the mean plus two standard deviations. The geometric mean and standard error of the geometric mean of titers for each group were calculated.

2.5. HSV-2 neutralization titers (ELVIS assay)

Individual sera (10 per group) were evaluated for HSV-2 neutralizing antibody by a colorimetric assay employing the ELVIS™ HSV cell line (Diagnostic Hybrids, Athens, OH) as described previously [6]. The neutralization titer was defined as the reciprocal of the greatest serum dilution that decreased the $OD_{570\,nm}$ obtained using the positive control (virus infected cells without antibody) by 50%. The geometric mean and standard error of the geometric mean of titers for each group were calculated.