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Liposomal gD ectodomain (gD_{1-306}) vaccine protects against HSV2 genital or rectal infection of female and male mice

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ABSTRACT

Herpes simplex virus type 2 (HSV2) is the most common causative agent of genital herpes, with infection rates as high as 1 in 6 adults. The present studies were done to evaluate the efficacy of a liposomal HSV2 gD_{1-306} vaccine (L-gD_{1-306}-HD) in an acute murine HSV2 infection model of intravaginal (female) or intrarectal (male or female) challenge. Two doses of L-gD_{1-306}-HD containing 60 μg gD_{1-306}-HD and 15 μg monophosphoryl lipid A (MPL) per dose provided protection against HSV2 intravaginal challenge (86–100% survival, $P \leq 0.0003$ vs. control liposomes; $P = 0.06$ vs. L-gD_{1-306}-HD without MPL). Both male and female mice (BALB/c and C57BL/6) immunized with L-gD_{1-306}-HD/MPL were significantly protected against HSV2 intrarectal challenge, with higher survival rates compared to controls (71–100%, $P \leq 0.007$). L-gD_{1-306}-HD/MPL also provided increased survival when compared to a liposomal peptide vaccine, L-$gD_{264-285}$-HD/MPL (male BALB/c, $P \leq 0.001$; female BALB/c and male C57BL/6, $P = 0.06$). Mice given L-gD_{1-306}-HD/MPL also had minimal disease signs, reduced viral burden in their spinal cords and elevated neutralizing antibody titers in the females. The vaccine also stimulated gD_{1-306}-HD specific splenocytes of both male and female mice with significantly elevated levels of IFN-γ compared to IL-4 ($P \leq 0.01$) indicating that there was an enhanced Th1 response. These results provide the first evidence that the L-gD_{1-306}-HD vaccine can protect both male and female mice against intrarectal HSV2 challenge.

1. Introduction

Herpes genitalis caused by Herpes simplex virus type 2 (HSV2) is one of the most common, sexually transmitted diseases in humans [1–4]. Epidemiological studies show that as many as 1 out of 6 Americans have been infected by HSV2 [3]. The disease affects both normal and immunosuppressed adults, and is associated with increased susceptibility to the human immunodeficiency virus [5,6]. Serious clinical disease can occur in neonates following transmission of virus from their infected mothers [7] who are also more likely to develop cervical cancer than non-infected women [4]. Thus, there is a critical need to develop an effective vaccine against this disease.

The most promising HSV2 vaccine targets have been the viral envelope glycoproteins [8], especially the 393 amino acid gD protein [9–11]. The gD envelope protein (59 kDa) (ectodomain 36.2 kDa) has been the focus of much attention because it has been shown to elicit neutralizing antibodies, and serves as a target for cellular immune responses in animal models [11–13] and more importantly, in humans [14]. The cellular immune response is believed to be a critical component for providing protection against both primary and recurrent disease [15–19]. In guinea pigs, immunization with gD has shown some success when given prophylactically, but is less effective in preventing the recurrence of lesions in already infected animals [20,21].

To date, the most successful vaccine candidate in clinical trials is composed of the gD protein and an adjuvant system (AS04), consisting of aluminum hydroxide and 3-*O*-deacylated-monophosphoryl lipid A (Simplirix®, manufactured by GlaxoSmithKline). This vaccine demonstrated significant efficacy against disease in women who were seronegative for HSV. However, it was not effective in men [22]. This suggests that there were gender differences in the immune responses to Simplirix® and underscores the need for preclinical testing of potential HSV2 vaccine candidates in both female and male animal models.

In previous studies [23], we observed significant protective effects in female mice challenged intravaginally with HSV2 following immunization with vaccines consisting of single epitopes derived from the HSV2 gD envelope protein and presented to the immune system by a highly immunogenic liposomal delivery vehicle. Within the gD envelope protein, a number of continuous small epitopes have been identified that are of potential vaccine interest because they are postulated to be located on protein surfaces, especially in regions where highly hydrophilic residues are present in

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beta turns [24–27], and they mediate antibody-dependent cellular cytotoxicity [28]. We hypothesized that the same type of liposomal delivery system that had been used for the single gD epitope vaccines could also be employed in the design of a vaccine incorporating the entire gD ectodomain (gD_{1-306}) and that the larger, potentially more immunogenic protein would stimulate a protective immune response in both female and male mice. In this study, we evaluated the efficacy of a liposomal formulation of gD_{1-306} in an intravaginal HSV2 murine model using different adjuvants, antigen doses and boosting schedules, compared its effectiveness in different mouse strains following HSV2 intrarectal challenge of male and female mice, and characterized the immune response of both sexes to the vaccine.

2. Materials and methods

2.1. Cloning and purification of HSV2 gD-HD protein

The nucleotide sequence was derived using optimized *Escherichia coli* codon preferences for the gD amino acid sequence. To prepare the $gD_{264-285}$ gene, two overlapping oligonucleotides (sense: 5′ CATATGACCCAGCCGGAACTGGTTCCGGAAGACCCGGAAGACTCCGCT and antisense: 5′ GTCGACGGTGCCAGCCGGGTCTTCCAGCAGAGCGGAGTCTTCCGGGTC) resulting in the gene product $gD_{264-285}$ (TQPELVPEDPEDSALLEDPAGT) were synthesized and flanked by convenient restriction sites (Nde I and Sal I underlined). Polymerase chain reaction (PCR) was used to amplify the overlapping oligonucleotides. One microgram of sense and antisense pair was incubated with 1× Pfx buffer, 2.5 mM dNTPs, 1 μL of Pfx polymerase (Invitrogen, San Diego, CA). The reaction mix was incubated using the following cycle 30 times: 94 °C for 1 min, ramp to 50 °C in 15 min, hold at 50 °C for 1 min, 72 °C for 1 min. Following PCR, the product was cleaved with Nde I and Sal I, and isolated by gel electrophoresis. The $gD_{264-285}$ gene was ligated into a similarly cleaved expression plasmid containing the proprietary hydrophobic domain (HD) gene (i.e., pET28a). The gD_{1-306} gene was created using the *E. coli* optimized sequence (BlueHeron Biotechnologies, Bothel, WA). The gD_{1-306} was subcloned into the pET28a vector using the Nde I and Sal I sites. The genes encoding $gD_{264-285}$ and gD_{1-306} were cloned upstream of the gene encoding the HD. The final genes were verified by sequencing the DNA. The gD_{1-306}-HD and the $gD_{264-285}$-HD containing plasmids were transformed into BL21 *E. coli* for expression. The bacteria were incubated in terrific broth medium with kanamycin (30 μg/mL) at 37 °C until mid-log phase and induced with 0.75 mM isopropyl-beta-D-thiogalactopyranoside (IPTG). The bacteria were harvested by centrifugation and the cell pellets lysed with 8 M urea/50 mM Tris–HCl pH 7.8 and centrifuged at 30,000 × g to remove cellular debris. The gD-HD proteins were purified by selective nickel affinity (Qiagen, Chatsworth, CA). Endotoxin was removed using detoxi-gel (Pierce, Rockville, IL). Endotoxin levels were determined by the Limulus Amebocyte Lysate assay (Cambrex Bioscience, Walkersville, MD). Protein purity and concentration were evaluated by Coomassie stain and the bicinchoninic acid (BCA) assay, respectively (Pierce, Rockville, IL).

2.2. Liposome preparation

The liposomes were prepared by dissolving the phospholipids, cholesterol, and monophosphoryl lipid A (MPL; Sigma, St Louis, MO) with recombinant gD_{1-306}-HD, recombinant $gD_{264-285}$-HD protein or without protein (control liposomes) in an organic solvent mixture of chloroform/methanol (1:1, v/v). The recombinant proteins with the HD were designed to stably integrate into the lipid bilayer of the liposomes. Thin lipid films were created by evaporating the solvent at 65 °C under a stream of nitrogen gas. The dried films were placed under vacuum for at least 24 h to remove residual organic solvent. Preparation of the liposomes was accomplished by hydrating the lipid films with buffer and incubating the suspension at 65 °C for 5–10 min before subjecting them to probe sonication. The liposomes were sterile filtered post-production through a 0.22 μm filter and sized by dynamic light scattering using a UPA-150 (Microtrac, North Largo, FL). To verify that the protein was incorporated in the liposomes, liposomes were passed over a size exclusion column (DG-10, Bio-Rad, Hercules, CA). Samples of the liposomes pre- and post-filtration were dissolved in 2.5% CHAPS detergent (Sigma–Aldrich, St. Louis, MO). The protein concentration was determined by the BCA assay (Pierce, Rockford, IL). The total encapsulated protein was greater than 95%.

2.3. Mice

BALB/c and C57BL/6 male and female mice (5–6 weeks) were purchased from Harlan Labs (Indianapolis, IN). All mice were housed and maintained in a pathogen-free environment, in accordance with the Animal Care and Use protocols at the California State Polytechnic University, Pomona vivarium.

2.4. Vaccination of mice

Six-week-old BALB/c or C57BL/6 mice ($n = 7$/group) were vaccinated subcutaneously (s.c.) on day 0 and day 56 (unless specified otherwise) with liposomes containing MPL and HSV2 gD amino acids 1–306 (L-gD_{1-306}-HD/MPL) or HSV2 gD amino acids 264–285 (L-$gD_{264-285}$-HD/MPL) or with liposomes containing only MPL (L-control/MPL) provided by Molecular Express, Inc. The mice were monitored for adverse reactions to vaccination including redness, swelling, or the formation of granulomas at the injection site. Mice were weighed daily for 1 week post-vaccination and weekly between the prime and boost.

2.5. Vaginal HSV2 infection of mice

At the time of boost (day −7 prior to challenge) and day −1 prior to challenge, mice were given a s.c. injection of Depo-Provera® (Pharmacia Corporation, MI) (2.67 mg medroxyprogesterone acetate/mouse). On the day of HSV2 challenge, mice were anesthetized intraperitoneally (i.p.) with ketamine (80 mg/kg) and xylazine (16 mg/kg) and the vagina of each mouse was swabbed with a Calcium Alginate Fiber Tipped Ultrafine Aluminum Applicator Swab (Fisher Scientific, PA) followed by intravaginal challenge with $50LD_{50}$ of HSV2 (strain G). Mice were weighed and observed for morbidity on a daily basis for 28 days. Vaginal scores were as follows: 0 = no lesion; 1 = erythema; 2 = mild inflammation; 3 = severe inflammation, fecal impaction or urinary incontinence; 4 = severe inflammation, epithelial tissue damage, fecal impaction and urinary incontinence. Neurological scores: 0 = no neurological signs; 1 = tail paralysis; 2 = tail and one hind leg paralyzed; 3 = tail and both hind legs paralyzed/hind quarter atrophy; 4 = moribund.

2.6. Rectal HSV2 infection of mice

Twelve hours prior to HSV2 challenge, food was withheld from the mice. On the day of challenge, mice were anesthetized i.p. with ketamine (80 mg/kg) and xylazine (16 mg/kg). The rectum of each mouse was washed 4× with 10 μL sterile PBS followed by 20 μL 2% nonoxynol-9 (Tergitol, Type NP-9, Sigma–Aldrich, MO) delivered into the rectum of each mouse and withdrawn after 5 min. Mice were intrarectally challenged with $10LD_{50}$ of HSV2 (strain G). The challenge dose for the intrarectal infection was lower than that for