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Inactivated HSV-2 in MPL/alum adjuvant provides nearly complete protection against genital infection and shedding following long term challenge and rechallenge

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ABSTRACT

Herpes Simplex Virus Type 2 (HSV-2) infection can result in life-long recurrent genital disease, asymptomatic virus shedding, and transmission. No vaccine to date has shown significant protection clinically. Here, we used a mouse model of genital HSV-2 infection to test the efficacy of a vaccine consisting of whole, formalin-inactivated HSV-2 (FI-HSV2) formulated with monophosphoryl lipid A (MPL) and alum adjuvants. Vaccine components were administered alone or as a prime-boost immunization together with DNA vaccines encoding a truncated glycoprotein D2 (gD2t) and two conserved HSV-2 genes necessary for virus replication, UL5 (DNA helicase) and UL30 (DNA polymerase). Our results show: (1) compared with mock immunized controls, mice immunized with FI-HSV2 plus MPL/alum consistently showed protection against disease burden and total viral shedding while the mice immunized with gD2t protein with MPL/alum did not; (2) protection against genital disease and viral replication correlated with the type of boost in a prime-boost immunization with little advantage afforded by a DNA prime; (3) intramuscular (i.m.) immunization with FI-HSV2 in MPL/Alhydrogel adjuvant provided nearly complete protection against vaginal HSV-2 shedding after a lethal intravaginal (i.vag.) short-term challenge and long-term rechallenge; (4) single formulation immunization with DNA vaccines, FI-HSV2, and MPL in an aluminum phosphate (Adju-Phos) adjuvant did not increase protection relative to FI-HSV2/MPL/Adju-Phos alone; and (5) addition of MPL/alum to the FI-HSV2 was required for optimal protection against disease, viral replication, and latent virus load in the dorsal root ganglia (DRG). Most notably, an optimized vaccine formulation of FI-HSV2 MPL/Alhydrogel given i.m. completely protected against detectable vaginal HSV-2 shedding in the majority of animals and HSV-2 latent DNA in the DRG of all animals.

1. Introduction

Herpes Simplex Virus Type 2 (HSV-2) is a common sexually transmitted infection [1]. It can cause serious disease in neonates [2] and immune compromised hosts [3] and is associated with increased risk of human immunodeficiency virus (HIV) acquisition [4,5]. HSV-2 replicates in the genital epithelium and is transported to the dorsal root ganglia (DRG) where it establishes a lifelong infection with reactivation and both symptomatic and asymptomatic shedding [6].

The correlates of protective immunity against HSV-2 are unknown. Studies in HSV-2/HIV-1 co-infected individuals have shown that CD4+ T cell loss correlates with increased HSV-2 shedding [7]. Neutralizing antibodies are important, but not sufficient in protecting against infection, as evidenced by failed vaccine trials [8]. During primary infection, CD8+ cytotoxic T cells may prevent acute ganglion infection [9], and reduce HSV-2 replication and shedding during recurrence [10,11]. Despite many strategies, no successful HSV-2 vaccine has yet been licensed [9]. A promising candidate, the GlaxoSmithKline (GSK) vaccine consisting of secreted gD2 protein formulated with monophosphoryl lipid A (MPL) and alum, did not show significant protection against HSV-2 infection or genital disease in the latest Phase 3 study, but did show efficacy against HSV-1 infection and genital

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disease [12]. Replication-defective virus vaccines for HSV-2 are also in the pipeline as they have been successful in animal models [13–17]. Chemically inactivated HSV-2 vaccines have been tested extensively in humans, but subsequently dismissed due to a lack of controls required for accurate data interpretation (reviewed in [18]). The data did show, however, the need for a durable immune response not elicited by inactivated virus alone [18]. Inactivated virus formulated with MPL/alum or other adjuvants, or given together with other vaccine platforms in a prime-boost combination, may provide the necessary enhanced immune responses.

We recently evaluated a DNA prime-inactivated virus boost strategy for its efficacy against intravaginal (i.vag.) HSV-2 challenge in guinea pigs, a model for studying both acute and recurrent disease [19]. Guinea pigs were primed with plasmids encoding gD2t, UL5, and UL30, and then boosted with formalin-inactivated HSV-2 (FI-HSV2) in MPL/Alhydrogel. After i.vag. challenge, this group showed a 97% reduction in recurrent lesion days compared with the mock controls, had the highest reduction in days with recurrent disease, and contained the lowest mean HSV-2 DNA load in the dorsal root ganglia. However, the FI-HSV2/MPL/Alhydrogel component alone (the pVAX empty vector DNA – FI-HSV2 group), with the exception of one "nonresponder" animal, elicited complete protection against both acute and recurrent lesions and detectable HSV-2 DNA in the DRG. Cumulative acute disease scores and numbers of recurrent disease days were also comparable to the UL5, UL30, and gD2t DNA-FI-HSV2 group.

In this report, we used the mouse model to further characterize the immunity and protection afforded by the DNA, FI-HSV2, and adjuvant. We also tested single formulation vaccines in order to simplify and expedite administration and to increase protective responses. Although HSV-2 does not spontaneously reactivate in mice, this i.vag. challenge model offers advantages in that it provides a lower cost system for rapidly evaluating vaccine candidates, has better characterized immunity to the virus with a wide variety of available reagents for the evaluation of specific T cell responses, has been optimized for progesterone treatment allowing for uniform susceptibility to HSV-2 infection, and allows the use of inbred, transgenic, and congenic strains. Together, experiments in the mouse model have demonstrated that protection against HSV-2 infection can be mediated by both cell mediated and humoral immunity.

2. Materials and methods

2.1. Vaccines

FI-HSV2 and a formalin inactivated mock preparation (FI-Mock) were prepared from HSV-2 infected or uninfected cells, respectively. Extracellular virus was purified and inactivated as previously described [19], and dextran sulfate wash-derived virus was purified as previously described for ELISA antigen [19] and then formalin inactivated. Cell associated virus was obtained from the clarified supernatant of HSV-2 infected Vero cells that were sonicated in virus containing extracellular media. The virus was subsequently pelleted and inactivated as described [19]. No infectious virus was detected after inactivation of any preparation. The preparation used for each experiment is denoted in each figure legend. All FI-HSV2 immunizations contained 10^7 PFU equivalents and 12.5 μg of MPL (Sigma L6895). Alum was purchased from Thermo Pierce (Imject alum) or Accurate Chemical & Scientific (Adju-Phos and Alhydrogel), with Al doses described in figure legends.

Plasmids expressing HSV-2 strain G UL5, UL30, and gD2t (aa 1–327) and the gD2t protein subunit vaccine were constructed and prepared as previously described [19].

2.2. Mice and ethics statement

Female BALB/c mice were purchased from Charles River Laboratories, housed in microisolator cages, and acclimated for at least 2 weeks prior to use. Studies were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All use of vertebrate animals was approved by the Institutional Animal Care and Use Committee, University of California, San Diego.

2.3. Intravaginal HSV-2 challenge model

Mice were treated with medroxyprogesterone acetate (Depo-Provera, Sigma M1629) [20] and i.vag. swabbed with a DPBS-moistened polyester tipped swab (MicroPur 1001D, PurFybr Solon, Rhinelander, WI) immediately prior to instillation of 5×10^4 PFU of HSV-2 strain G (*ca.* 10 LD_{50} doses) by micropipette. The HSV-2 preparation used for challenge was described previously [19].

Anogenital disease was scored as described in Fig. 1 legend. Vaginal virus shedding of infectious virus was measured by plaque assay [19], and the limit of sensitivity of each assay is denoted in each figure.

2.4. Antibody quantification

2.4.1. HSV-2 virion specific IgG

ELISAs were as previously described [19] except that alkaline phosphatase goat anti-mouse conjugates specific for IgG (Sigma), IgG1 or IgG2a (SouthernBiotech) were used, and endpoint titers were Fit Spline interpolated (GraphPad Prism 5.0d). Specifically, plates were coated overnight with 50 μl per well of either live, dextran sulfate-released HSV-2 (2.35×10^6 PFU) or an equal protein mass of uninfected Vero cell sonicate (0.1 μg), with subsequent UV-treatment of coated HSV-2 plates. Pre-challenge serum samples from individual mice were initially diluted 1:40 and then serially diluted 4-fold to 655,360 for analysis.

2.4.2. Neutralizing antibody assay

Complement-dependent HSV-2 neutralizing antibodies were measured by plaque reduction assay as described previously [21].

2.5. Intracellular cytokine staining

Eleven days following the second injection, mice were *in vivo* restimulated by HSV-2 injection in the footpad. Four days later, splenocytes were stimulated *in vitro* with 10 PFU per cell of HSV-2 (or an equivalent volume of a mock preparation) for 2 h. Brefeldin A was added for an additional 8 h.

For staining, a viability dye (LIVE/DEAD fixable violet; Molecular Probes, Invitrogen) and Fc block (CD16/32; BD) was added for 30 min at 4 °C. Antibodies to surface markers CD8 (CD8-Ax488; Clone 53-6.7; BD) and CD4 (CD4-Ax647; clone RM4-5; BD) were added for 30 min at 4 °C and then cells permeabilized and fixed using the BD Cytofix/Cytoperm kit. Antibodies to CD3 (CD3-PE-Cy5; Clone 145-2C11; BD) and IFN-γ (IFN-γ-PE; Clone XMG1.2; BD) were included in the intracellular stain for 45 min at 4 °C. Data from 50,000 to 100,000 live CD3+ T cells were collected on a BD FACSCanto flow cytometer and analyzed with BD FACSDiva software at the Research Flow Cytometry Core Facility of the San Diego Center for AIDS Research and the Veterans Medical Research Foundation and VA San Diego Healthcare System, La Jolla, CA.

2.6. Quantification of HSV-2 DNA in DRG

Four week postchallenge lumbosacral DRG from each surviving mouse and 4 naïve mice were removed, pooled, and frozen.