Vaccine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Intramuscular vaccination of guinea pigs with the live-attenuated human herpes simplex vaccine VC2 stimulates a transcriptional profile of vaginal Th17 and regulatory Tr1 responses

Brent A. Stanfield, Paul J.F. Rider, John Caskey, Fabio Del Piero, Konstantin G. Kousoulas *

Department of Pathobiological Sciences and Division of Biotechnology and Molecular Medicine, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, USA

ARTICLE INFO

Article history:
Received 17 July 2017
Received in revised form 23 March 2018
Accepted 27 March 2018
Available online xxxx

Keywords:
Herpes simplex virus
Live-attenuated
IL17
Th17
Pathogenesis
Innate immune response
Genital infection
Vaccine

ABSTRACT

Herpes simplex virus is a common causative agent of oral and genital diseases. Novel vaccines and therapeutics are needed to combat herpes infections especially after the failure of subunit vaccines in human clinical trials. We have shown that the live-attenuated HSV-1 VC2 vaccine strain is unable to establish latency in vaccinated animals and produces a robust immune response capable of completely protecting mice against lethal vaginal HSV-1 or HSV-2 infections. The guinea pig represents the best small animal model of genital HSV-2 disease. Reported here, twenty-one female Hartley guinea pigs received intramuscular injection with either the VC2 vaccine, or equal volume of conditioned tissue culture media. Animals received 2 booster vaccinations at 21 day intervals following the initial vaccination. After vaccination, animals were challenged with the highly virulent HSV-2 (G) strain. Histologically, VC2 vaccinated animals had little to no apparent inflammation/disease following challenge. Unvaccinated animals developed moderate to severe erosive and ulcerative vaginitis. Quantitative reverse-transcriptase PCR analysis in VC2 vaccinated and challenged animals identified transcriptional signatures of Th17 and regulatory Tr1 cells associated with the inflammatory response primed by VC2 vaccination. Treatment of cultured human vaginal epithelial cells (VK2 cells) with a combination of IL-17A and IL-22 resulted in the significant induction of beta-defensin 3 expression. Further, treatment of VK2 cells with IL-17A, IL-22, IL-36 or beta-defensin 3 resulted in diminished HSV-2 replication. Overall, these results suggest that intramuscular vaccination with the live-attenuated vaccine VC2 primes a mucosal immune response predisposing the adaptive expression of transcripts associated with a Th17 response to challenge and these responses contribute to antiviral immunity.

1. Introduction

Human herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2 respectively) are common human pathogens that are typically associated with infection of the oral and genital mucosa. Infection with either of these viruses results in a life-long latent infection with sporadic reactivation and shedding of infectious virus. Herpes associated disease can manifest pathologically as blistering and ulceration at the initial site of infection, but more severe and debilitating pathologies can manifest as permanent stromal keratitis of the cornea and meningoencephalitis [1]. HSV-2 infection has a high global prevalence. Recent estimates from the world health organization show that approximately 536 million individuals were living with HSV-2 infection in 2003 with 23.6 million new cases in the same year [2]. In the United States, increased prevalence of HSV-2 infection is significantly associated with lower income [3]. Data from a study conducted between 2003 and 2013 indicated that the incidence of HIV/HSV-2 co-infection was significantly increased, while both HIV infections and hepatitis C/HIV co-infections declined. There were no significant changes observed in the seroprevalence of HSV-2 through the same time period indicating that sexual practices were not changed. Overall, these data suggest that HSV-2 infection is a significant cofactor for HIV infection [4].

Previously, our laboratory has shown that the live-attenuated herpes simplex VC2 vaccine strain is safe and effective at preventing genital HSV-1 and HSV-2 disease in mice. The VC2 vaccine contains 2 truncating mutations in the genes coding for glycoprotein K (gK) and the membrane protein UL20 [5]. The 37 amino acid deletion in the amino terminus of gK results in a virus incapable of entering into the axonal termini of cultured neurons [6]. While

* Corresponding author.
E-mail address: vtgusk@lsu.edu (K.G. Kousoulas).

https://doi.org/10.1016/j.vaccine.2018.03.075
0264-410X/

the 18 amino acid deletion in UL20 is attenuating the exact mechanism has yet to be established (Stanfield and Kousoulas, unpublished observations). Despite the majority of preclinical herpes vaccine research being conducted utilizing mice [reviewed in: [7]], very few vaccine candidates progress from the preclinical to the clinical environment and currently no vaccine has proven effective at preventing disease in humans. Unfortunately, mice are not optimal models for this disease, largely because they do not accurately depict immunological and inflammatory characteristics of disease observed in humans [8–10]. In contrast, guinea pigs offer a model of genital HSV-2 disease closely mimicking human disease [8,11–13]. Recently, our laboratory has demonstrated that the VC2 vaccine is safe and immunogenic in rhesus macaques [14]. In macaques VC2 is capable of stimulating the expansion of vaginal $CD4^+$ and $CD8^+$ T cells and germinal center responses resulting in the sustained production of highly cross neutralizing antibodies against HSV-1 and HSV-2 after intramuscular vaccination [14].

A variety of evidence in experimental animals and humans indicate that both humoral and cellular immune responses are responsible for providing protective immunity against genital herpes [7]. In the murine model of genital HSV-2 infection, it has been reported that $CD4^+$ T cells played a critical role in preventing disease [15], most likely by clearing infectious virus at neural sites via a non-lytic mechanism [16]. Recently, the importance of IL-17A in the adaptive response to genital HSV-2 infection has been demonstrated in mice [17]. T helper 17 cells (Th17) are a subset of pro-inflammatory $CD4^+$ T cells defined by their production of interleukin 17 (IL-17). Th17 cells also secrete cytokines such as IL-17A, IL-17F, IL-21, and IL-22. These cytokines act through the IL-17 receptor (IL-17R) found on the surface of a variety of cell types including epithelial cells. Interaction of IL-17A with IL-17R initiates a signaling cascade through ACT1 and TRAF6 to stimulate the production of proinflammatory IL-36 family cytokines [reviewed in: [18]]. These IL-36 family cytokines and specifically IL-36γ then signal via the IL-36 receptor to stimulate the secretion of antimicrobial peptides [19]. Genital Th17 responses are known to be important for the clearance of bacterial pathogens [10,20–22]. A variety of evidence suggests that Th17 cells play important roles in vaccine-induced protection against a variety of pathogens entering the host via mucosal surfaces [23,24]. Recently estradiol has been shown to prime vaginal dendritic cells to induce Th17 responses demonstrating increased vaccine efficacy with estradiol treatment against genital HSV-2 challenge and IL-17 knock-out mice were more susceptible to HSV-2 challenge [25].

To understand the immune response generated by intramuscular injection with the VC2 vaccine, we utilized the guinea pig to model genital HSV-2 infection. The guinea pig is widely regarded as the best small animal model of genital HSV-2 disease. Herein, we show that the live-attenuated HSV-1 VC2 vaccine strain induces mucosal IL-17A responses that contribute to protection against lethal challenge with virulent HSV-2 through the induction of the antiviral peptide beta-defensin 3.

2. Materials and methods

2.1. Ethics statement

This work was approved by the Louisiana State University School of Veterinary Medicine IACUC protocol number 14-040. The Office of Laboratory Animal Welfare of the National Institutes of Health (NIH) has approved LSU regarding the use of animals in research with an approval Assurance Statement (#A3612-01).

2.2. Cells and viruses

Green African Monkey Kidney cells (Vero) (ATCC, CCL-81) were maintained in DMEM (ThermoFisher) containing 50 mg Primocin (ThermoFisher) and 10% heat inactivated fetal bovine serum (FBS) (ThermoFisher). Stock HSV-2 (G) was grown to high titer and titrated in confluent monolayers of Vero cells. The live-attenuated VC2 vaccine strain was described previously, as described previously [5]. Human vaginal mucosa epithelial cells (VK2/E6E7) (ATCC, CRL-2616) were cultured in Keratinocyte-SFM (ThermoFisher) containing 1% heat inactivated FBS (ThermoFisher).

2.3. Preparation of vaccine

Serum-free stock VC2 virus for multiple injections was prepared by infection of Vero cells at an MOI of 0.001 followed by replacement of culture media with serum free DMEM (ThermoFisher). Infection was allowed to progress for 48 h and then virus was collected and aliquoted for administration to the animals.

2.4. Vaccination and challenge scheme

Twenty-one, 250–300 g, female Hartley guinea pigs (Charles River) were housed in groups of 3 per cage. Groups were then divided randomly into the three treatment groups. Guinea pigs were mildly anesthetized by inhalation of 2–3% isoflurane prior to vaccination and were euthanized by CO_2 asphyxiation.

A total of 21 animals were used in this study. They were divided in three groups as follows: Group 1 had three animals that were left unvaccinated and unchallenged; group 2 had nine animals that received intramuscular injection with conditioned media; group 3 had nine animals that were vaccinated with the VC2 virus. On day 0 of the study animals received intra-muscular injection of either 100 μL of conditioned media (unvaccinated), or 100 μL of serum free media containing 2×10^6 PFU of VC2 vaccine. Animals received booster vaccines on days 21 and 42 post initial inoculation. On day 63 of the study, animals to be challenged were lightly anesthetized by inhalation of 2–3% isoflurane and then inoculated intravaginally with HSV-2 (G strain) by first clearing the mucus plug from the vagina with a cotton swab, followed by a second cotton swab inside the vaginal vault to further dry the walls of the vagina, and finally, using a micropipette, instilling the vaginal vault with 40 μL complete DMEM containing 1×10^6 PFU of HSV-2 (G).

2.5. Tissue collection and euthanasia

At 1 h, 3 days, and 8 days post challenge, animals were anesthetized by inhalation of 2–3% isoflurane and bled via cardiac puncture. Maximum volume of blood was collected and animals were euthanized by 2 asphyxiation. Vaginal tissue samples were collected immediately following euthanasia, placed in RNAlater (Ambion) and stored at −20 °C until use.

2.6. Histology

All animals were examined for the identification of macroscopic lesions. All tissues and organs were collected and fixed in 10% buffered formalin. Sections of tissues from the genital tracts and lymphoid organs were dehydrated in progressive alcoholic solutions and xylene and paraffin embedded. Five micron tissue sections were obtained, lined on glass slides, stained with hematoxylin and eosin and examined with light microscopy.

Please cite this article in press as: Stanfield BA et al. Intramuscular vaccination of guinea pigs with the live-attenuated human herpes simplex vaccine VC2 stimulates a transcriptional profile of vaginal Th17 and regulatory Tr1 responses. Vaccine (2018), https://doi.org/10.1016/j.vaccine.2018.03.075