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Short communication

Antibodies to ovine herpesvirus 2 glycoproteins decrease virus infectivity and prevent malignant catarrhal fever in rabbits

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ABSTRACT

Ovine herpesvirus-2 (OvHV-2) is the etiological agent of sheep-associated malignant catarrhal fever (SA-MCF), a fatal lymphoproliferative disease of many species in the order Artiodactyla. Development of a vaccine is critical to prevent mortality. Because OvHV-2 has not been cultured *in vitro*, SA-MCF research is hindered by the lack of *in vitro* tools to study viral constituents and specific host immune responses. As an alternative, in this study the neutralizing activity of antibodies against OvHV-2 glycoproteins gB and gH/gL was evaluated *in vivo* using rabbits. OvHV-2-specific antibodies were developed in rabbits by immunization using biolistic delivery of plasmids expressing the genes of interest. A lethal dose of OvHV-2 was incubated with the antisera and then nebulized into rabbits. Virus neutralization was assessed by measuring infection parameters associated with the virus infectious dose. Anti-gB or anti-gH/gL antibodies alone blocked infection in five out of six rabbits (83%), while a combination of anti-gB and anti-gH/gL antibodies protected all six rabbits (100%) from infection. These results indicate that antibodies to OvHV-2 gB and gH/gL are capable of neutralizing virions, and consequently, reduce virus infectivity and prevent SA-MCF in rabbits. Thus, OvHV-2 gB and gH/gL are suitable targets to be tested in a SA-MCF vaccine aimed at stimulating neutralizing antibody responses.

1. Introduction

Ovine herpesvirus 2 (OvHV-2), a gammaherpesvirus in the genus *Macavirus*, is carried asymptomatically by sheep, its well-adapted natural host. Infection with OvHV-2 causes sheep-associated malignant catarrhal fever (SA-MCF) in non-adapted hosts such as cattle, bison, deer, pigs and other species in the order Artiodactyla (O'Toole and Li, 2014). SA-MCF is a fatal lymphoproliferative disease of significant agricultural impact worldwide. American bison are highly susceptible to SA-MCF (Gailbreath et al., 2010; Li et al., 2014; O'Toole and Li, 2014) and the disease is a major concern of the bison industry in North America, where outbreaks result in loss of an appreciable number of animals (Berezowski et al., 2005; Li et al., 2006;

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Schultheiss et al., 1998). Separation of clinically susceptible species from sheep is currently the only means available to reduce transmission risk of OvHV-2 and the occurrence of SA-MCF, yet this is not always practical.

An effective vaccine for SA-MCF is needed. However, the absence of an *in vitro* system to culture OvHV-2 and the consequent lack of tools to study OvHV-2 components has hindered the process of vaccine development. Methods to attenuate or manipulate OvHV-2 are currently not viable. Therefore, common tools used to study the function of viral proteins and host antibody responses are also deficient or absent. Molecular mechanisms for OvHV-2 infection are poorly characterized, but based on other herpesviruses, it is known that glycoproteins gB, gH and gL are conserved among herpesviruses and constitute the core components responsible for viral entry (Connolly et al., 2011). Neutralizing epitopes in gB and in the heterodimer gH/gL have been identified in gammaherpesviruses, even though the neutralizing efficiency of specific antibodies varies (Chesnokova and Hutt-Fletcher, 2011; Gill et al., 2006; Gillet et al., 2006; Naranatt et al., 2002). The presence of neutralizing epitopes in gB, gH and gL suggests that these proteins would be logical candidates to be targeted in a virus-blocking vaccine. In this study, the neutralizing activity of antibodies specific to the OvHV-2 gB, gH and gL was evaluated *in vivo*, using a recently developed system to measure the ability of antibodies to block OvHV-2 infection in rabbits (Li et al., 2013). Our major goal was to determine whether anti-OvHV-2 gB, gH and/or gL antibodies are capable of virus neutralization at initial infection, resulting in decreased viral infectivity and prevention of disease.

2. Methods

2.1. Animals

Thirty-six 3-month-old New Zealand white rabbits were used in the study. Six rabbits were used for production of antisera to OvHV-2 gB, gH and gL, and an additional 30 rabbits were used in the virus-blocking experiments. The animals were maintained at Washington State University, Pullman, WA in accordance with the legal requirements of the relevant local or national authority and approved animal care and use protocols.

2.2. OvHV-2 gB, gH and gL expressing plasmids

OvHV-2 gB, gH and gL glycoproteins are encoded by the genes identified as open reading frame (ORF) 8, ORF 22, and ORF 47, respectively, in the virus genome (Genbank: DQ198083). Codon-optimized sequences of these three genes were synthesized by GeneArt® (Life Technologies). The Gateway® Technology System (Life Technologies) was used for cloning and protein expression; pDONOR™221 and pcDNA™3.2/V5-DEST were used as entry and expression vectors, respectively. Cloning procedures were performed according to manufacturer's recommendations. Large scale preparations of the expression plasmids were performed to obtain high quality, endotoxin-free plasmid DNA, suitable for *in vitro* and *in vivo* cell transfection (EndoFree Plasmid Mega Kit, Qiagen). Protein expression was confirmed by transfecting Chinese Hamster Ovary (CHO) cells with the expression plasmids *via* lipid-mediated transfection (Attractene Transfection Reagent, Qiagen), in accordance with the manufacturer's recommendations. Cells were harvested at 24 hours post-transfection, lysed (Cell Lysis Buffer, Promega) and the protein extracts subjected to immunoblotting to detect the V5 epitope tag fused to the C-terminus of the proteins of interest. Protein extracts were treated under reducing (50 mM dithiothreitol and 10 min at 70 °C) or non-reducing (no reducing agent and no heat) conditions and separated using SDS-PAGE (NuPAGE® 4-12% Bis-Tris Gel, Life Technologies). Proteins were transferred from the gel onto a nitrocellulose membrane and probed with mouse anti-V5-HRP antibody (Life Technologies) diluted at 1:5000. Chemiluminescent reagents (HyGlo Quick spray™, Denville Scientific) and X-ray film (Kodak) were used to detect binding of the V5-HRP antibody.

2.3. Antisera production

To produce antisera against the OvHV-2 gB, gH and gL, rabbits were immunized with the constructed expression plasmids using biolistic DNA delivery to the skin (Helios® Gene Gun System, Bio-Rad). DNA-coated bullets were designed to deliver 1 μg of DNA per shot. The cartridges were prepared according to the manufacturer's instructions (Bio-Rad) using 1 μm gold particles, 0.05 mg of polyvinylpyrrolidone mL^{-1}, a Microcarrier Loading Quantity (MLQ) equal to 0.5 mg per shot and a DNA Loading Ratio (DLR) of 2 μg of DNA per mg of gold. Rabbits were anesthetized prior to immunizations using isofluorane and then DNA-coated gold particles were bombarded on shaved abdominal skin at 400 psi helium pressure. Immunizations consisted of three (for gB groups) or four (for gH/gL group) DNA deliveries at 3-week intervals. Four rabbits (R1 to R4) were immunized with the gB plasmid DNA, rabbits R1 and R2 received a total of 24 μg of DNA per immunization while rabbits R3 and R4 received a total of 12 μg of DNA per immunization. For the gH/gL immunization, two rabbits (R5 and R6) received 18 μg of each plasmid per delivery. Because gH/gL bullets delivered 0.5 μg of each plasmid, the number of immunizations and amount of DNA delivered to rabbits R5 and R6 was adjusted to achieve the same dose of each plasmid (72 μg) delivered to rabbits R1 and R2, immunized with the gB plasmid (1 μg of DNA per bullet), during the course of immunizations. Between one and three weeks after the final immunization serum was collected by cardiac puncture as a terminal procedure before euthanasia.

Following immunizations, rabbits were monitored for antibody production by ELISA. Assays specific for gB and gH/gL were performed using protein extracts, prepared from CHO cells transfected with the respective plasmids as described above. The amount and concentration of each reagent was optimized by checker-board titration. Briefly, 96-well plates (Immulon® 2 HB Flat Bottom MicroTiter®) were coated with the optimal dilution of antigen and incubated at 4 °C for 12–16 hours. Unbound antigen was removed, plates were blocked (20% milk, 0.02% tween