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Short communication

A DNA vaccine against dolphin morbillivirus is immunogenic in bottlenose dolphins

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Abstract

The immunization of exotic species presents considerable challenges. Nevertheless, for facilities like zoos, animal parks, government facilities and non-profit conservation groups, the protection of valuable and endangered species from infectious disease is a growing concern. The rationale for immunization in these species parallels that for human and companion animals; to decrease the incidence of disease. The U.S. Navy Marine Mammal Program, in collaboration with industry and academic partners, has developed and evaluated a DNA vaccine targeting a marine viral pathogen – dolphin morbillivirus (DMV). The DMV vaccine consists of the fusion (F) and hemagglutinin (H) genes of DMV. Vaccine constructs (pVR-DMV-F and pVR-DMV-H) were evaluated for expression in vitro and then for immunogenicity in mice. Injection protocols were designed for application in Atlantic bottlenose dolphins (*Tursiops truncatus*) to balance vaccine effectiveness with clinical utility. Six dolphins were inoculated, four animals received both pDMV-F and pDMV-H and two animals received a mock vaccine (vector alone). All animals received an inoculation week 0, followed by two booster injections weeks 8 and 14. Vaccine-specific immune responses were documented in all four vaccinated animals. To our knowledge, this is the first report of pathogen-specific immunogenicity to a DNA vaccine in an aquatic mammal species.

Keywords: Morbillivirus; DNA vaccine and dolphin

1. Introduction

The United States Navy maintains five Marine Mammal Systems (MMS). These MMS consist of pinniped and cetacean species (California sea lions and bottlenose dolphins) that are deployed in oceans throughout the world. These animals are trained to perform specialized tasks, including mine-hunting and swimmer defense, and are therefore invaluable to the Navy. Due to the nature of their employment, these animals face possible exposure to infectious disease agents in their working environment, both at home and abroad. Minimizing the potential impact of infectious disease on these animals is therefore a priority objective of the Navy Marine Mammal Program's (NMMP) preventive medicine efforts. While active immunization represents the best means with which to provide protection against infectious disease, there are currently no vaccines licensed for use in cetacean and pinniped species. Moreover, there is no consensus among

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specialists on which modality can provide both adequate safety and efficacy across a broad spectrum of pathogen targets in both species. Nevertheless, the vaccination of these animals remains an important preventive medicine mandate and a feasible goal despite the unique environmental and immunological settings.

The rationale for the development of vaccines for use in these animals is straightforward. However, both the uniqueness of these species and their specialized vocation present several challenges that influence our choice of modality, as well as our ability to evaluate these vaccine candidates in vivo. Initially, our challenge was to determine which vaccine modality represented the greatest combination of attributes. The potential for the development of adverse effects attributable to certain immunization modalities is an unacceptable risk for this population. In addition, any candidate vaccine must be able to induce both humoral and cellular immunity to provide protection against a potentially broad range of pathogen targets. Gene-based vaccines may provide an advantage in this respect. DNA vaccines induce balanced immunity, generating both humoral and cellular responses (Donnelly et al., 1997; Robinson et al., 1993; Ulmer et al., 1993), and studies in numerous animal models and in human clinical trials have shown this modality to be safe (Donnelly et al., 1997; Prince et al., 1997; Sarzotti et al., 1997; Martinez et al., 1997; Pavlenko et al., 2004; Ada and Ramshaw, 2003). Gene-based immunization therefore represents a tool with which to potentially provide a high degree of immunogenicity and formulative flexibility, with the greatest level of safety for these untested species.

As part of NMMP's disease prophylaxis and surveillance efforts several infectious disease agents have been identified as potential health threats to Navy animals. Morbillivirus (Paramyxoviridae) has now been identified as a significant cause of disease outbreaks and mortality in marine mammal species around the world (de Swart et al., 1995; Hall, 1995, Trends Microbiol; Visser et al., 1993). Epizootics of these viruses have been responsible for mass mortality events in marine mammals belonging to the orders Pinnipedia (seals) and Cetacea (whales, porpoises and dolphins), and have occurred in widely dispersed locations (Mahy et al., 1988; Van Bressem et al., 2001; Domingo et al., 1990; Duignan et al., 1997; Lipscome et al., 1994; Taubenberger et al., 1996). Sympatric species and cross-species infections are now known to be common, making it difficult to distinguish the true prevalence and therefore the real level of threat to the Navy's animals should they deploy to areas where morbillivirus has occurred and/or is endemic.

The design and development of a DNA vaccine targeting dolphin morbillivirus (DMV) was undertaken through collaboration with industry and academic partners. The vaccine formulation consisted of two viral gene targets: the fusion protein (F) and the hemagglutinin protein (H) of DMV. Both genes have well-established roles in immunogenicity and pathogenesis within the Paramyxoviridae family (Atabani et al., 1997; Ertl et al., 2003; Partidos et al., 1999). The objective was to test the candidate plasmid DMV vaccine in a small group of Navy dolphins in order to evaluate its immunogenicity. Previous dose-escalation studies conducted in collaboration with Vical, Inc. established the safety of plasmid DNA injections in Navy dolphins (Smith CR Proceedings, 2002). A total of six dolphins were immunized with plasmid DNA, four animals received pVR-DMV-F and pVR-DMV-H, and two animals received mock-vaccine (pVR vector alone). The results of this study demonstrate some level of vaccine-specific humoral and cellular responses in all vaccinated animals, and suggest a potential for future use in this species.

2. Methods and materials

2.1. Animals

Six adult bottlenose dolphins (*Tursiops truncatus*). This group included three males and three females, ranging in age from 16 to 46 years. All animals were housed in open ocean enclosures at the U.S. Navy Marine Mammal Program's facility in San Diego, California. All animal related work was in accordance with the NMMP's Institutional Animal Care and Use Committee (IACUC) guidelines. The NMMP laboratory is an Association for the Accreditation and Assessment of Laboratory Animal Care International (AAALAC) approved facility.

2.2. Preparation of plasmid DNA

The full-length fusion and hemagglutinin genes of dolphin morbillivirus (provided by Tracey Schock, University of Georgia) were sub-cloned into the Sal I and Not I sites of the eukaryotic expression vector pVR-1055 (provided by Vical, Inc.). The pVR-DMV-F and pVR-DMV-H constructs were then propagated in *E. coli*, and transformants were selected based on their growth in the presence of kanamycin. Restriction enzyme analysis and DNA sequencing were performed to confirm the correct orientation and identity of the inserted genes. Constructs were prepared for injection using alkaline/SDS lysis, followed by two rounds of CsCl-EtBr gradient ultracentrifugation. Each plasmid