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Research paper

Cellular immune responses in cetaceans immunized with a porcine erysipelas vaccine

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ABSTRACT

Clinical erysipelas represents a significant health problem in managed cetacean species. Vaccination was suspended in many oceanariums in the past due to losses associated with vaccine-induced hypersensitivities which were deemed to be a greater threat than clinical erysipelas. A perceived shift in clinical presentation of erysipelas from a chronic dermatologic form to an acute systemic form in dolphins sparked interest in re-initiating vaccination with improved subunit vaccines of *Erysipelothrix rhusiopathiae*. This manuscript describes the development and application of *in vitro* correlates of immunity (T_H1, T_H2 and T_{REG}) in *Tursiops truncatus* induced by immunization with a commercial porcine 65 kDa subunit *E. rhusiopathiae* vaccine. Variable degrees of pre-existing T cell memory were identified prior to vaccination. Vaccine-induced IFNγ responses were consistent with a T_H1 response and associated with elimination of erysipelas in all vaccinated animals. Comparative analysis between six-month and 12-month vaccination booster regimes demonstrated maintenance of superior memory in the six-month group; however, anamnestic responses induced by booster were only identified in the 12-month group. To our knowledge, this is the first study to develop and apply advanced immunologic analyses for assessing vaccine efficacy in captive or free-ranging wildlife.

1. Introduction

Erysipelothrix rhusiopathiae, the causative agent of erysipelas, has continued to be an important recognized pathogen for cetaceans since it was first reported in 1956 in the spotted dolphin (*Stenella plagiodon)* and Atlantic bottlenose dolphin (*Tursiops truncatus*) (Seibold and Neal, 1956; Wood, 1999). Erysipelas has since been reported in other cetacean species including the Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), Indian ocean bottlenose dolphin (*Tursiops aduncus*), Risso dolphin (*Grampus griseus*), white-beaked dolphin (*Lagenorhunchus albirostris*), beluga whale *(Delphinapterus leucas*), and killer whale (*Orcinus orca*) (Buck and Spotte, 1986; Calle et al., 1993; Dierauf and Gulland, 2001; Geraci et al., 1966; Thurman et al., 1983; Wood and Shuman, 1981). The primary route of transmission of *E. rhusiopathiae,* a Gram-positive, intracellular bacteria, is believed to be ingestion of bacteria-laden fish (Geraci et al., 1966; Wood and Shuman, 1981); however, it has been suggested that select dolphins can be carriers of the bacteria, through chronic, low level infection.

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Two forms of erysipelas have been described in cetaceans, a septicemic form and a dermatologic form; both are similar to those observed in infected swine (Dierauf and Gulland, 2001; Wood, 1984). Signs of septicemic disease in infected cetaceans are usually of a non-specific nature and not unique to erysipelas; death within a few hours of clinical presentation is not unusual (Dierauf and Gulland, 2001; Medway, 1980; Sweeney and Ridgway, 1975). The dermatologic form of the disease occurs periodically and is typically accompanied by a leukocytosis and anorexia with subsequent development of skin lesions, the latter being most commonly visible on the trunk (Medway, 1980; Sweeney and Ridgway, 1975). Septicemia and death can occur within days or weeks if gone untreated. Immediate administration of antibiotics to suspected cases minimizes animal fatalities; such treatment is often initiated before *E. rhusiopathiae* infection is confirmed (Dierauf and Gulland, 2001). However, antibiotic-mediated early termination of infection could compromise generation of T cell-mediated immunity, leaving the animal susceptible to re-infection (Tseng et al., 2009).

The value of implementing a vaccination program for erysipelas became apparent with the observation that the majority of clinical cases, recorded between 1989 and 2000, were in cetaceans that had not been vaccinated (Lacave, personal communication via Dierauf and Gulland, 2001); cetaceans on a regular vaccine schedule showed no disease expression (Dierauf and Gulland, 2001). While a variety of vaccines (modified-live and bacterin) have been available for many years, a recent survey of 75 oceanariums (Lacave, personal communication via Dierauf and Gulland, 2001) identified that only approximately 1/3 were actively vaccinating against *E. rhusiopathiae* (Dierauf and Gulland, 2001). While the efficacy of most vaccines appeared adequate, widespread reports of adverse clinical reactions to vaccination, including deaths, had been reported (Dierauf and Gulland, 2001; Geraci et al., 1966; Howard et al., 1983; Ridgway, 1972). Such adverse side effects were considered to be of greater concern than clinical erysipelas and prompted many oceanariums to discontinue *E. rhusiopathiae* cetacean vaccination programs.

A recent shift of erysipelas disease expression from the dermatologic to the septicemic form was observed in a private collection of cetaceans beginning in 2000 (Jim McBain; unpublished observation). Five clinical cases of chronic dermatologic erysipelas were recorded in this population of 200 cetaceans between 1985 and 2000; no clinical cases of acute systemic infections were identified. Subsequently, eight clinical cases of acute and often fatal systemic erysipelas, and one case of the chronic dermatologic form, were identified in the same cetacean population between 2000 and 2009. This perceived shift towards acute fatal erysipelas stimulated many oceanariums to consider re-initiating vaccination programs. The new generation porcine vaccine used in the current study is based upon the 64–67 kDa (p65) bacterial surface protein which is thought to be involved in the induction of protective immunity to *E. rhusiopathiae* (John and Timoney, 1993; Kobayashi et al., 1992; Sato et al., 1995; Yamazaki et al., 1999).

Essential to the implementation of any vaccine program is demonstration of vaccine efficacy and safety. Live pathogen challenge with *E. rhusiopathiae* is not feasible, relative to establishing efficacy of this porcine erysipelas vaccine in cetaceans. This manuscript describes the development and application of a model system for establishing *in vitro* cellular correlates of erysipelas vaccine efficacy in marine mammals. The focus was vaccine-induced expansion of antigen-specific T_H1 and/or T_H2 lymphocyte memory. Relative levels of mRNA transcripts encoding interferon gamma (IFNγ) served as a correlate of a T_H1 response while IL-4 and IL-13 served as correlates for T_H2 type responses. T_H1 responses are associated with cell-mediated immunity (CMI) and elimination of intracellular pathogens while T_H2 responses are typically associated with elimination of helminthic pathogens and immediate-type hypersensitivities; an extensive review of these and other functional T lymphocyte subsets has recently been published (Zhu et al., 2010).

2. Materials and methods

2.1. Animals and vaccination

Dolphins were vaccinated with ER Bac® Plus (Pfizer Animal Health, Exton, PA), as per manufacture's instructions for swine. Atlantic bottlenose dolphins (*Tursiops truncatus*) involved in this study were housed at three Sea World Adventure Parks in the United States. Three study groups were employed sequentially to address the following: (i) response of adult dolphins to primary immunization with the vaccine, (ii) relative duration of T cell memory (six-compared to 12-month vaccination regimes) and (iii) response of juveniles to primary vaccination.

Study #1 consisted of 22 unvaccinated dolphins ranging in age from three to six years (Table 1). These animals were selected for immunologic analysis based upon their availability for obtaining follow-up blood samples two weeks following the 2nd vaccination (administered three weeks following initial vaccination). Five animals were selected from Park 1, 13 animals from Park 2, and four from Park 3. All animals were observed for adverse reactions following vaccinations (local clinical reactions at the site of vaccination, clinical signs of anaphylaxis and alterations in animal behavior including appetite). Negative control dolphins were not available for this study as the total population was logically assumed to be naturally exposed to *E. rhusiopathiae* through their feed (frozen fish); the presence of the bacteria in the commercially purchased frozen fish had been previously documented. Two previously unvaccinated dolphins, both from Park 2, had developed clinical erysipelas. A six-year-old adult male (#0211) had a case of acute systemic erysipelas (study #1) and a six-month-old juvenile male #3017 developed dermatologic erysipelas (study #3). No mature dolphins were left unvaccinated due to the urgent and immediate need to reduce clinical erysipelas in the population.

Study #2 included 26 dolphins (ranging in age from four to 40 years) to compare the response to recall antigen in animals that received booster vaccinations every six months versus those receiving boosters at 12-month intervals (Table 2). Animals had previously received two to eight vaccinations (Table 2). Thirteen animals from Park 2