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Clinical and immunological assessment of therapeutic immunization with a subunit vaccine for recurrent ocular canine herpesvirus-1 infection in dogs

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ABSTRACT

Latent canine herpesvirus-1 (CHV-1) infections are common in domestic dogs and reactivation of latent virus may be associated with recurrent ocular disease. The objectives of the present study were to evaluate the ability of a subunit CHV-1 vaccine to stimulate peripheral CHV-1 specific immunity and prevent recurrent CHV-1 ocular disease and viral shedding. Mature dogs with experimentally-induced latent CHV-1 infection received a 2-dose CHV-1 vaccine series. Recurrent ocular CHV-1 infection was induced by corticosteroid administration in the prevaccinal, short-term postvaccinal (2 weeks post-vaccination), and long-term postvacccinal (34 weeks post-vaccination) periods. Immunological, virological, and clinical parameters were evaluated during each study period. Quantitative assessment of peripheral immunity included lymphocyte immunophenotyping, proliferation response, and interferon-γ production; and CHV-1 virus neutralizing antibody production. In the present study, vaccination did not prevent development of ocular disease and viral shedding; however, there was a significant decrease in clinical ocular disease scores in the short-term postvaccinal period. Significant alterations in peripheral immunity detected in the dogs during the short-term and long-term postvaccinal periods included increased T and B lymphocyte subpopulation percentage distributions, increased lymphocyte expression of major histocompatibility complex class I and II, increased CHV-1 virus neutralizing antibody titers, decreased lymphocyte proliferation, and decreased interferon-γ production. Vaccination of latently infected mature dogs with the selected subunit CHV-1 vaccine was not effective in preventing recurrent ocular CHV-1 infection and viral shedding induced by corticosteroid administration. The vaccine did induce long-term CHV-1 specific immunity and may decrease the severity of clinical ocular disease in the immediate postvaccinal period.

1. Introduction

Canine herpesvirus-1 (CHV-1) is an alphaherpesvirus with a host range restricted to canids. First identified in the 1960s as an etiologic agent of systemic fetal and neonatal infections in domestic dogs, CHV-1 is now also appreciated as an ocular, respiratory, and genital pathogen of mature dogs (Evermann et al., 2011; Carmichael et al., 1965). In contrast to neonatal infections, CHV-1 diseases in mature dogs may be associated with either primary or recurrent viral infection (Evermann et al., 2011). During primary CHV-1 infection, virus is transported in sensory nerves by retrograde axonal transport to regional sensory ganglia where it establishes latency (Miyoshi et al., 1999). Latent CHV-1 infections are endemic in domestic dog populations world-wide (Krogenaes et al., 2012; Yesilbag et al., 2012). When latent CHV-1 reactivates in the sensory ganglia, anterograde neuronal transport of virus back to peripheral tissue sites is associated with viral replication, viral shedding, and potentially recurrent disease (Ledbetter et al., 2012; Okuda et al., 1993). Ocular lesions in mature dogs associated with primary and recurrent CHV-1 infection include blepharitis; conjunctivitis; conjunctival ulcerations; punctate, dendritic, and geographic ulcerative keratitis; and nonulcerative keratitis (Ledbetter, 2013).

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As is typical of many of the alphaherpesviruses, naturally-acquired primary and recurrent CHV-1 infection does not elicit a sufficient immune response to prevent future reactivations of latent virus in some dogs (Ledbetter et al., 2012; Okuda et al., 1993). Seroprevalence studies indicate a high frequency of latent CHV-1 infection in many canine populations, and that primary infection often occurs at a young age in dogs (Krogenaes et al., 2012; Yesilbag et al., 2012). These epidemiologic features suggest that prophylactic vaccination of seronegative dogs to prevent primary CHV-1 infection, and possibly establishment of latency, would not be feasible or applicable in many dogs. In contrast, therapeutic vaccination to prevent viral reactivation or ameliorate clinical disease and viral shedding associated with recurrent CHV-1 infection could be useful. This vaccinal strategy would be applied to specific canine populations to reduce the risk of viral transmission between dogs or used in individual dogs with a history of recurrent CHV-1 infection and at risk for recrudescent CHV-1 diseases (Gervais et al., 2012; Ledbetter et al., 2006).

A subunit CHV-1 vaccine (Eurican Herpes 205, Merial, Lyon, France) is commercially available in some countries for the immunization of CHV-1 seronegative pregnant dams to prevent or reduce morbidity and mortality in their puppies with acquired CHV-1 infection in the immediate postpartum period. The clinical efficacy of the vaccine for protecting neonatal dogs from mortality associated with CHV-1 infection was demonstrated in an experimental infection study and field trials (Chabchoub et al., 2006; Poulet et al., 2001). The efficacy of the vaccine in the prevention of CHV-1 disease and viral shedding in latently infected mature dogs is not reported. The objectives of the present study were to evaluate the ability of the subunit CHV-1 vaccine to stimulate peripheral CHV-1 specific immunity and prevent recurrent CHV-1 disease and viral shedding in latently infected mature dogs. Using an established recurrent CHV-1 ocular disease model, the duration of vaccinal response and protection was also evaluated using clinical, immunological, and virological outcome measures.

2. Materials and methods

2.1. Animals and induction of latent CHV-1 infection

All protocols were approved by the Animal Care and Use Committee of Cornell University and were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Ten 1.5-year-old, specific pathogen-free Beagles were used including 8 intact males and 2 intact females. All dogs were seronegative for CHV-1 prior to experimental infection. Dogs were maintained individually in runs and direct contact between dogs was prevented for the duration of the study. Strict bioisolation was maintained throughout the study for all personnel in contact with dogs. The dogs were maintained in the isolation facilities for the duration of the study. Dogs were acclimated to the housing facilities for a minimum of 4 weeks prior to the beginning of the study.

Latent CHV-1 infection was experimentally induced in each dog by topical ocular inoculation 10 weeks prior to the beginning of the study using the ocular drop method as previously described (Ledbetter et al., 2009). Dogs were topically inoculated in both eyes with 2×10^5 $TCID_{50}$ of a field strain of CHV-1 isolated from corneal samples of a dog with dendritic ulcerative keratitis treated at the Cornell University College of Veterinary Medicine Hospital for Animals (Ithaca, NY, USA), followed immediately by gentle manual massage of the closed eyelids for 60 s.

2.2. Study design

Total study duration was 345 days and divided into 3 separate investigative periods: prevaccinal, short-term postvaccinal, and long-term postvaccinal (Fig. 1). During the initial 30 study days, prevaccinal immunity and baseline viral reactivation data were collected. Dogs then received a 2-dose vaccine series, with the vaccine administered to each dog on study days 35 and 77. Postvaccinal immunity was assessed on study days 91 (short-term postvaccinal period) and 315 (long-term postvaccinal period). Following immune assessments, postvaccinal viral reactivations were induced beginning on study days 92 and 316, and the impact of vaccination on clinical disease and viral shedding was evaluated for the subsequent 30 days.

2.3. Prevaccinal immunity and viral reactivation

Ten weeks after recovery from primary ocular infection, the presence of reactivatable CHV-1 latency was experimentally confirmed as previously described (Ledbetter et al., 2012) by administration of an immunosuppressive systemic dose of prednisolone (3.0 mg/kg, PO, q 24 h) to the dogs for 7 consecutive days.

One day prior to prednisolone administration, blood was collected for immunologic assessment, complete blood count (CBC), and serum biochemistry panel (SBP). Immunologic

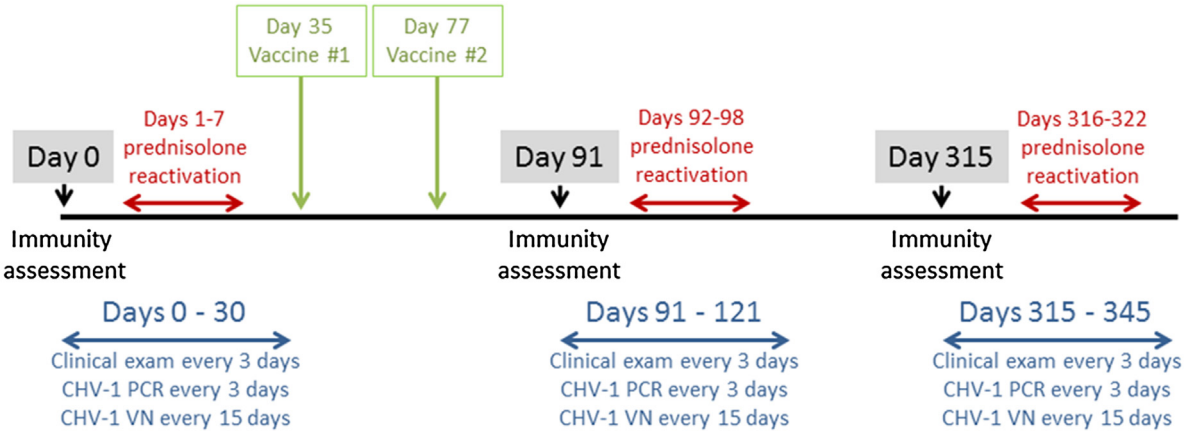

Fig. 1. Basic study design flow chart. The study was divided into 3 investigative periods: prevaccinal, short-term postvaccinal, and long-term postvaccinal. The prevaccinal (baseline) period spanned days 0–30. The short-term postvaccinal (2 weeks after completing the vaccine series) spanned days 91–121. The long-term postvaccinal (34 weeks after completing the vaccine series) spanned days 315–345.