



Safety and serological response to a matrix gene-deleted rabies virus-based vaccine vector in dogs



James P. McGettigan^{a,b,*}, Frederic David^c, Monica Dias Figueiredo^c, Jules Minke^d, Teshome Mebatsion^c, Matthias J. Schnell^{a,b}

^a Department of Microbiology and Immunology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA, United States

^b Jefferson Vaccine Center, Thomas Jefferson University, Philadelphia, PA, United States

^c Merial-1730 Olympic Drive, Athens, GA 30601, United States

^d Merial, 254 rue Marcel Mérieux, 69007 Lyon, France

ARTICLE INFO

Article history:

Received 21 October 2013

Received in revised form 6 January 2014

Accepted 15 January 2014

Available online 7 February 2014

Keywords:

Canine

Rabies

Replication-deficient RABV

Vaccine

ABSTRACT

Dogs account for the majority of human exposures and deaths due to rabies virus (RABV) worldwide. In this report, we show that a replication-deficient RABV-based vaccine in which the matrix gene is deleted (RABV-ΔM) is safe and induces rapid and potent VNA titers after a single inoculation in dogs. Average VNA titers peaked at 3.02 or 5.11 international units (IU/ml) by 14 days post-immunization with a single dose of 10^6 or 10^7 focus forming units (ffu), respectively, of RABV-ΔM. By day 70 post immunization, all dogs immunized with either dose of vaccine showed VNA titers >0.5 IU/ml, the level indicative of a satisfactory immunization. Importantly, no systemic or local reactions were noted in any dog immunized with RABV-ΔM. The elimination of dog rabies through mass vaccination is hindered by limited resources, requirement for repeat vaccinations often for the life of a dog, and in some parts of the world, inferior vaccine quality. Our preliminary safety and immunogenicity data in dogs suggest that RABV-ΔM might complement currently used inactivated RABV-based vaccines in vaccination campaigns by helping to obtain 100% response in vaccinated dogs, thereby increasing overall vaccination coverage.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Controlling rabies in domesticated or feral dogs is considered one of the most effective approaches to protect humans from rabies infection. In Central and South America, evidence suggests that continued mass vaccination of dogs helped to eliminate or contain rabies in that region of the world [1,2]. Nonetheless, over 90% of human rabies exposures, and over 99% of human rabies deaths, are still due to rabid dogs [2–5].

While the elimination of domesticated dog rabies through mass vaccination is considered feasible [2], limited resources, inferior vaccine quality in parts of the world, particularly in China, and low immunization coverage are considered important factors that hinder effective rabies control [5]. When mass vaccination campaigns are implemented, they are often shown to be effective in achieving the WHO recommended rate of $>70\%$ canine vaccination rate [6]. However, the turnover of the dog population, and the requirement

for continual boost inoculations for the life of the dog, translates into millions of vaccinations or revaccinations every year [4]. In some parts of the world, mass vaccination campaigns are not effective and programs would need to be implemented every 1 to 6 years to achieve the WHO recommended canine vaccination rate [7]. Other regions of the world, such as in some rural areas of China where only 2–8% of the dogs are vaccinated [5], require mass vaccination programs to be initiated. To achieve the goal for improved and continued mass vaccination programs on a global scale, public health assistance and the availability of lower cost vaccine strategies are required [2,7]. One potential way to reduce the burden of public health dollars spent on the control of rabies virus in dogs is to reduce the number of inoculations required to protect each dog.

We previously showed that a replication-deficient RABV-based vaccine in which the matrix gene is deleted (RABV-ΔM) is immunogenic in mice and nonhuman primates [8–10]. Furthermore, RABV-ΔM is apathogenic and does not spread to the CNS of T and B cell-deficient mice [8]. Due to the safety and immunogenicity profile of RABV-ΔM, we tested the safety and antibody responses in dogs immunized with a single dose of RABV-ΔM. We show that RABV-ΔM is safe in dogs and results in potent anti-RABV immunity, indicating that RABV-ΔM may help reduce the burden of canine rabies by helping to enhance vaccination coverage of pets and feral

* Corresponding author at: Department of Microbiology and Immunology, Thomas Jefferson University, Jefferson Alumni Hall, Suite 466, Philadelphia, PA 19107, United States. +1 215 503 4629.

E-mail address: james.mcgettigan@jefferson.edu (J.P. McGettigan).

dogs and potentially reducing the need for repeated inoculations for protection.

2. Materials and methods

2.1. Test vaccine

The replication-deficient, matrix gene-deleted rabies virus-based vaccine (rRABV-ΔM) used in these studies was cloned, recovered and characterized as described previously [8]. Vaccine stocks were grown on BSR cells (a baby hamster kidney cell line) stably expressing RABV M (BSR-RABVM) [8,11] in DMEM supplemented with 1% fetal bovine serum (FBS) for four days. Supernatants were collected and clarified by centrifugation at 900g, 10 min. Aliquots of the virus stock were frozen at -80°C until used. Virus was titered in triplicate using BSR-RABV M cells. Virus dilution were made in PBS on the day of immunization and kept on ice until administered. The virus stock was tested for sterility using general-purpose media. No growth was detected in fluid thioglycollate medium or trypticase soy broth (plus agar) inoculated with 100 μl virus stock and incubated at room temperature or at 35°C . Growth was detected in the positive control samples of fluid thioglycollate or trypticase soy broth at both temperatures; no growth was observed in the negative control using the same media at the same incubation temperatures.

2.2. Animals

Male and female canines (Closed Colony Mongrels; Liberty Research, Inc.) between the ages of 4–13 months were used. No history of rabies vaccination were noted and confirmed negative by Rapid Fluorescent Focus Inhibition Test (RFFIT) prior to the onset of the study [<0.2 international units (IU)/ml]. Dogs were acclimated to housing for at least 7 days prior to the start of the study. Animals were determined to be healthy and no clinical signs of disease were noted prior to vaccination. Animals were handled in compliance with Merial Institutional Animal Care and Use Committee (IACUC) approvals.

2.3. Immunization and sample collection

Dogs were divided into two groups of 5 dogs/group: Groups A and B dogs received a single dose of 10^6 or 10^7 ffu/ml of vaccine, respectively, in a volume of 1 ml administered subcutaneously in the shoulder area. Dogs were monitored intermittently for 1 h post-vaccination for any systemic adverse events, and injection site observations and rectal temperatures were taken on days 0, 1, 2, and 3 post-immunization. Blood was collected on days 7, 14, 28, 49, and 70 post-immunization and then processed for serum, divided into two aliquots and the serum stored frozen (-20°C) until testing for serology testing.

2.4. Immune assays and statistical analysis

Serum rabies antibody titers were quantified using RFFIT [10,12–14]. Personnel performing the laboratory tests were blind to the treatment groups. A titer value less than or equal to the assay detection limit (0.2 IU/ml) was replaced by 0.2 IU/ml for all analyses. Statistical analyses were conducted using SAS v9.1 Enterprise Guide (SAS Institute, Cary, NC). All tests were two sided and statistical significance was declared at a P value of 0.05 or less.

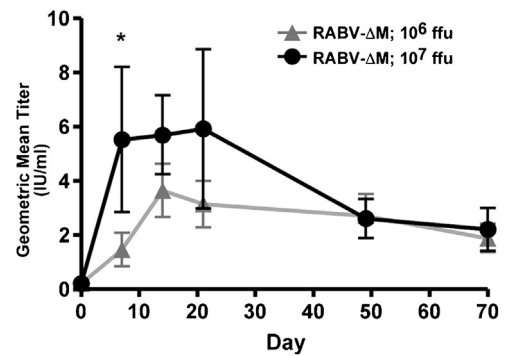


Fig. 1. Kinetics analyses of VNA responses in dogs immunized with RABV-ΔM. Neutralization titers, defined as the inverse of the highest serum dilution that neutralizes 50% of the challenge virus (Challenge Virus Strain 11), were normalized to international units/ml (IU/ml) using the WHO anti-RABV antibody reference standard. Virus neutralizing antibodies peaked on average around day 14 post-immunization and all dogs maintained VNA titers greater than 0.5 IU/ml for the duration of the study. Except for day 7, antibody titers were similar for each dilution at the indicated time points (* P value <0.05). Error bars represent the standard error of the mean (SEM).

3. Results

3.1. Immunization of dogs with RABV-ΔM does not result in systemic or local adverse events

Five 4–13 month-old mongrel dogs per group were injected subcutaneously with 10^6 or 10^7 ffu of RABV-ΔM. No vaccination site reaction, pain upon palpation or injection site swelling was observed in either group. Rectal temperatures remained within normal limits for the duration of the study. In one dog (Group B), a rectal temperature of 40.4°C was recorded one day post-vaccination. However, this hyperthermia was probably not related to vaccination since this dog had a pre-vaccination baseline rectal temperature of 40.3°C . Dogs vaccinated with 10^6 or 10^7 ffu of RABV-ΔM showed no clinical signs of disease for the duration of the study (i.e., 70 days post-injection). Together, the preliminary preclinical testing shows that RABV-ΔM does not cause systemic or local adverse events in immunized dogs.

3.2. Immunization with RABV-ΔM results in long lasting effective anti-RABV immunity in dogs

Serum rabies antibody titers were quantified using RFFIT from blood collected on days -29 , 7, 14, 28, 49 and 70, relative to the day of vaccination. A VNA titer of 0.5 IU/ml is indicative of a satisfactory immunization of dogs by regulatory agencies from most areas of the world currently considered “rabies free” [15–17]. As shown in Table 1 and Fig. 1, animals immunized with either 10^6 or 10^7 ffu/dog showed VNA titers greater than 0.5 IU/ml by 14 days post-immunization, which corresponded to the peak average VNA titers in both groups of dogs. VNA titers then declined to a set point approximately two to eight times the indicative level of a satisfactory immunization at 70 days post-immunization. While peak VNA titers were higher in dogs immunized with 10^7 ffu compared to dogs immunized with 10^6 ffu, similar kinetics and longer-term VNA titers were detected in dogs immunized with 10^6 or 10^7 ffu of vaccine. No vaccine failures were noted in any animal. Together, this data indicates that RABV-ΔM is highly immunogenic, safe, and induces potent anti-RABV immunity in dogs.

4. Discussion

The ability for current inactivated RABV-based vaccines to induce protective immunity in dogs relies on many host- and

Download English Version:

<https://daneshyari.com/en/article/2402254>

Download Persian Version:

<https://daneshyari.com/article/2402254>

[Daneshyari.com](https://daneshyari.com)