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One-time intradermal DNA vaccination in ear pinnae one year prior to infection protects dogs against rabies virus

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Abstract

Rabid dog exposures result in >99% of human rabies deaths worldwide. Ninety-eight percent of these cases occur in developing countries. Thus, the best protection against human rabies would be prevention through adequate vaccination of the reservoir population. The difficulty in re-locating ownerless, freely roaming dogs for booster vaccinations, in addition to poor coverage with inadequate vaccines, suggests that a potentially inexpensive vaccine that elicits long-term protection after a single-dose could improve control of canine rabies in developing countries. One solution could be a DNA vaccine. We have previously determined that dogs vaccinated intradermally (i.d.) in ear pinnae with a rabies DNA vaccine expressing a rabies virus glycoprotein (G) produce high levels of neutralizing antibody that persist for at least 6 months. In the present study, we determined whether a one-time i.d. rabies DNA vaccination into ear pinnae 1 year before viral challenge would protect dogs against rabies virus. The dogs did not receive a booster vaccination. All dogs (100%) vaccinated i.d. in each ear pinna with 50 μg of rabies DNA vaccine, or intramuscular (i.m.) with a commercial canine rabies vaccine survived a lethal dose of rabies virus. In contrast, 100% of dogs vaccinated i.m. with 100 μg of rabies DNA developed rabies, as did 100% of negative control dogs that were vaccinated i.d. in each ear pinna or i.m. with DNA that did not express the rabies virus G. The data suggest that a one-time i.d. rabies DNA vaccination into ear pinnae offers a new approach to facilitate control of endemic canine rabies in developing countries.

Keywords: Rabies; Dogs; One-time DNA vaccination; 100% long-term protection; Ear pinna

1. Introduction

Dogs have transmitted rabies to humans since the disease was recognized in antiquity. The “mad dog” was mentioned with respect and concern in the legal documents of Mesopotamia in the 23rd century B.C., and ancient Chinese writings indicate that rabies was recognized in dogs centuries before the birth of Christ [1]. Presently, >99% of human rabies deaths world-wide are the result of exposure to rabid dogs [2]. Ninety-eight percent of the cases occur in the developing countries of Asia, Africa and Latin America [3,4]. It is estimated that at least 50 million dogs are vaccinated each year against rabies in either private practices or during national campaigns organized by ministries of health or agriculture. Unfortunately, in developing countries where dogs are the viral reservoir, the 30–50% vaccination coverage of canine populations is insufficient to break the disease transmission cycle [5]. The poor vaccination coverage with sometimes inferior vaccines that fail to maintain persistent levels of neutralizing antibody, in addition to the difficulty in re-locating ownerless, free-roaming dogs for booster vaccinations, suggests that a potentially inexpensive rabies vaccine that elicits long-term protection after a one-time vaccination

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might facilitate control of canine rabies in developing countries. A solution could be a DNA vaccine.

Earlier studies have determined that DNA vaccines administered prophylactically or therapeutically are effective in protecting mice against rabies virus [6–10]. Rabies DNA vaccination also protects against rabies virus challenge neonatal mice that possess maternal anti-rabies virus antibody or passively-transferred rabies hyper-immune serum [11]. Furthermore, rabies DNA vaccination protects 100% of nonhuman primates against a viral challenge that kills 100% of negative control monkeys [12]. It also has been shown that Beagle dogs vaccinated with a rabies DNA vaccine produce neutralizing antibody [13]. We expanded upon that study showing that an i.d. injection of DNA into ear pinnae of Beagle dogs is an optimal route for elicitation of elevated levels of neutralizing antibody that persist for at least 6 months [14]. Lastly, others have determined that dogs vaccinated i.m. with a rabies DNA vaccine, and then boosted with the same vaccine 56 days before viral challenge are protected against a wild-type dog rabies virus [15]. Dogs receiving only a primary DNA vaccination were not included in the study.

This study was designed to determine whether one dose of a rabies DNA vaccine administered 1 year before viral challenge would protect Beagle dogs against rabies. The 100% survival of dogs vaccinated in each ear pinna suggested that a one-time i.d. DNA vaccination might be considered as a means to aid in control of canine rabies in developing countries.

2. Materials and methods

2.1. Plasmid construction

Construction of the rabies DNA vaccine using the pCMV4 plasmid encoding the glycoprotein (G) of the CVS rabies virus has been previously described [6].

2.2. Beagle dogs

Thirty female Beagle dogs (12–14 months of age) ranging in weight from 7.6–10.8 kg were purchased from Ridglan Farms Inc., P.O. Box 318, Horeb, WI 53572. The dogs had previously received vaccination against canine distemper virus, adenovirus type 2, leptospirosis, canine parvovirus, canine papilloma, bordetella, and canine parainfluenza virus, but had not been vaccinated against rabies. All dogs were healthy, and were group housed indoors in artificial light at the Ridglan Farms facility. The dogs, randomly assigned to experimental groups, were vaccinated with the vaccines tested in this study on site at Ridglan Farms, exercised daily, fed commercial dog chow and received water ad libitum. Eleven months after vaccination the dogs were shipped via commercial cargo truck to the Rabies Section, Center for Disease Control and Prevention (CDCP), Lawrenceville, GA. The dogs were housed individually at CDCP. One month after arrival at CDCP the dogs were challenged with rabies virus. The animal facilities and animal care and use programs at Ridglan Farms and CDCP are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Both function in accordance with all United States Department of Agriculture, Department of Health and Human Services Regulations and Standards.

2.3. Vaccinations

The dogs were placed in six groups of five each and vaccinated one time as follows: Group 1—i.d. in each ear pinna with 50 μg rabies DNA/0.1 ml; Group 2—i.d. in the left ear pinna with 100 μg rabies DNA/0.1 ml; Group 3—i.d. (0.1 ml) in each ear pinna with 50 μg of pCMV4 control DNA that did not express the rabies virus G; Group 4—i.m. in the left quadriceps muscle with 100 μg rabies DNA/0.1 ml; Group 5—i.m. (0.1 ml) in the left quadriceps muscle with 100 μg of pCMV4 control DNA that did not express the rabies virus G; Group 6—i.m. (1.0 ml) in the left quadriceps muscle with the canine rabies commercial vaccine RabVac 3 (Fort Dodge Animal Health, Fort Dodge, IA). The dogs did not receive a booster vaccination. The dogs were bled prior to vaccination, bi-monthly for 1 year, 7 days after viral challenge and either at the time of euthanasia (non-protected), or 90 days after viral challenge (survivors).

2.4. Rabies virus challenge

Dogs were challenged with rabies virus 382 days after vaccination. Five-tenths milliliter of a 1:5 dilution of a salivary gland homogenate obtained from a rabid dog naturally infected with a coyote rabies virus variant [16] was injected into each masseter muscle. The viral titer of the stock salivary gland homogenate was $10^{6.5}$ mouse intracranial lethal $doses_{50}$/0.03 ml. A similar challenge with the same concentration of the identical virus preparation caused rabies in 100% of non-vaccinated negative control dogs. After viral challenge, the dogs were observed several times daily for clinical signs associated with rabies. At the first definitive clinical signs, animals were sedated and euthanatized with a barbiturate solution administered intravenously. At necropsy, brain impressions were made and tested for rabies viral antigen by the direct fluorescent antibody test [17].

2.5. Neutralizing antibody assay

Serum anti-rabies virus neutralizing antibody titers were performed as previously described using the rapid fluorescent focus inhibition test (RFFIT) [12]. The data will be illustrated as titers of individual sera or of the geometric mean (GM) of sera in each group of dogs that reduced the number of fluorescent foci 50%. Anti-sera of known international units (IU)/ml of rabies virus neutralizing antibody, a rabies hyperimmune mouse serum, and the United States Standard Human Rabies Immunoglobulin R2, were included as positive controls in