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ORIGINAL ARTICLE

Intranasal Anti-rabies DNA Immunization Promotes a Th1-related Cytokine Stimulation Associated with Plasmid Survival Time

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Background and Aims. DNA vaccination has a great potential to decrease infectious diseases worldwide, such as rabies. Here we showed the effects of a single anti-rabies DNA vaccination applied intranasally (IN) on plasmid survival time, neutralizing antibody (NA) titers, G-protein expression and Th1/Th2-related cytokines.

Methods. Only one 50-μg dose of an anti-rabies DNA vaccine was IN administered to 160 Balb/c mice. Twenty mice were used for the neutralizing antibody study, 35 for the proliferation assay, 35 for Th1/Th2-related cytokines, 35 for glycoprotein expression by immunocytochemistry, and 35 for pGQH detection and G-protein mRNA expression.

Results. Th1-type related cytokines from spleen cells (IFN-γ, TNF-α, and IL-2) were detected. Rabies NA titers were ≥0.6 IUs from day 30 onward in the IN DNA-vaccinated group. The plasmid was identified in brains and lungs from days 3–15. The mRNA transcript was amplified in brains and lungs from days 3–30, and G-protein expression was observed in spleens, brains and lungs on days 3, 8, and 15. In all cases, a gradual decrease was observed on days 30 and 45 and absent on day 60.

Conclusions. We found that Th1-type related cytokines (IL-2, IFN-γ, and TNF-α) were stimulated during the first month after DNA vaccination, correlating with the proliferation assays. Also, it was associated with the plasmid survival time remaining in lungs and brains prior to its degradation.

Key Words: Th1 immune response, Intranasal, DNA vaccine, Rabies, pGQH, Central nervous system.

Introduction

Rabies is an acute and mortal infection that affects the central nervous system (CNS) in warm-blooded animals including humans (1). The World Health Organization (WHO) has reported that ~55,000 people die from rabies every year (2).

Vaccination prevents rabies virus infection, being the most effective medical tool available to reduce mortality and as post-exposure prophylaxis (PEP) once the infectious agent has entered the body. Actually, tissue-culture vaccines such as purified chick embryo cell vaccine (PCECV) have been shown to provide protection against

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Table 1. Groups of animals according to the test performed

	Neutralization antibody study	Proliferation assays	Th1/Th2-related cytokines	Glycoprotein expression by IHC	pGQH and G protein mRNA expression
Number of mice	20	35	35	35	35
Number of groups	2 (n = 10)	7 (n = 5)	7 (n = 5)	7 (n = 5)	7 (n = 5)
Day of sacrifice	60	0,3,8,15,30,45,60	0,3,8,15,30,45,60	0,3,8,15,30,45,60	0,3,8,15,30,45,60

IHC, immunohistochemistry.
Number of mice per group (n).

classical rabies virus (RABV) via pre-exposure or PEP, being the best available alternative today.

Rabies virus has the highest fatality rate of all known human viral pathogens with a handful of exceptions. Humans who develop a symptomatic rabies virus infection inevitably die. Alternative treatments for rabies virus infections in humans currently are not safe (3,4).

Titers ≥0.5 international units (IUs), as determined by an infectious foci reduction assay against a WHO reference serum, are considered protective in all mammalian species tested to date (2).

The advantages of genetic vaccines over traditional ones are well established (5–7). In this sense, DNA vaccines are safe. They do not integrate into the host cell genome or induce autoimmune diseases or antibodies to DNA, and they only elicit mild local reactions as shown in numerous clinical trials (8).

These characteristics make genetic vaccines an excellent option, especially in developing countries (9–12).

IN immunization has been recognized as an effective route for the induction of humoral and cellular immune responses. This method results in a substantial absorption of DNA that is subsequently distributed and expressed in different sites throughout the organism, including lymph nodes (13,14).

Little is known about the Th1/Th2-related cytokines following IN vaccination with an anti-rabies DNA vaccine. Here we studied the Th1/Th2-related cytokines from spleen cells, neutralizing antibody (NA) titers, plasmid survival time, and G-protein expression in the mouse lung and brain.

Materials and Methods

Animals

For this study we used 160 (8- to 10-week-old) Balb/c mice from The Jackson Laboratory (Bar Harbor, ME). Twenty mice were used for the NA study, 35 for the proliferation assay, 35 for Th1/Th2-related cytokines, 35 for the glycoprotein expression by immunocytochemistry, and 35 for pGQH and G-protein mRNA expressions (Table 1). Mice were housed in filter-top cages and provided with sterile food and water ad libitum at the Animal Facility of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ). This study was performed in accordance with the Guidelines for the Care and Use of Laboratory Animals from the Office of Laboratory Animal Welfare. The protocol for animal use was approved by the Institutional Animal Care and Use Committee of the INCMN SZ.

Neutralizing Antibody Production

Two groups of ten mice each were formed to study NA production by the fluorescent focus reduction rapid test (FFRRT): group A received 50 μL PBS by IN administration and was used as control; group B received 50 μg of DNA vaccine diluted in 50 μL of phosphate-buffered saline (PBS) by IN administration. Blood samples were taken from all animals to obtain serum before starting the experiment (day 0) and later on days 15, 30, 45, and 60 after inoculation. Sera were stored at −20°C until tests were performed. All mice were sacrificed at day 60 after obtaining the last blood sample.

Proliferation Assay

Seven groups of five mice each were formed to study the proliferation assay. The control group received 50 μL of PBS IN, 72 h prior to sacrifice on day 0. The experimental groups received the IN DNA vaccine on day 0. One group of mice was sacrificed per date on days 3, 8, 15, 30, 45, and 60. Three days before the first sacrifice (day 0), experimental groups received 50 μg of DNA vaccine diluted in 50 μL PBS.

Th1/Th2-Related Cytokines

Seven groups of five mice each were formed to study the Th1/Th2-related cytokines. The control group received 50 μL of PBS IN, 72 h prior to sacrifice on day 0. The experimental groups received the IN DNA vaccine on day 0. One group of mice was sacrificed per date on days 3, 8, 15, 30, 45, and 60. Three days before the first sacrifice (day 0), experimental groups received 50 μg of DNA vaccine diluted in 50 μL PBS.

G-protein Detection

Seven groups of five mice each were formed to study glycoprotein expression in the mouse spleen, brain and lungs. The control group received 50 μL of PBS administered IN, 72 h prior to sacrifice on day 0. The experimental