Fish & Shellfish Immunology 37 (2014) 220–228

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Food pellets as an effective delivery method for a DNA vaccine against infectious pancreatic necrosis virus in rainbow trout (*Oncorhynchus mykiss*, Walbaum)

Natalia A. Ballesteros, Sylvia Rodriguez Saint-Jean, Sara I. Perez-Prieto*

Dept. Microbiología Molecular y Biología de las infecciones, Centro de Investigaciones Biológicas (CSIC), C/Ramiro de Maeztu 9, 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 27 November 2013
Received in revised form
7 February 2014
Accepted 9 February 2014
Available online 21 February 2014

Keywords:
Food DNA vaccine
Infectious pancreatic necrosis virus
Oral pcDNA.VP2 vaccine
Fish viruses
Rainbow trout

ABSTRACT

A DNA vaccine based on the VP2 gene of infectious pancreatic necrosis virus (IPNV) was incorporated into feed to evaluate the effectiveness of this oral delivery method in rainbow trout. Lyophilized alginate–plasmid complexes were added to feed dissolved in water and the mixture was then lyophilized again. We compared rainbow trout that were fed for 3 consecutive days with vaccine pellets with fish that received the empty plasmid or a commercial pellet. VP2 gene expression could be detected in tissues of different organs in the rainbow trout that received the pcDNA-VP2 coated feed (kidney, spleen, gut and gill) throughout the 15 day time-course of the experiments. This pcDNA-VP2 vaccine clearly induced an innate and specific immune-response, significantly up-regulating IFN-1, IFN-γ, Mx-1, IL8, IL12, IgM and IgT expression. Strong protection, with relative survival rates of 78%–85.9% were recorded in the vaccinated trout, which produced detectable levels of anti-IPNV neutralizing antibodies during 90 days at least. Indeed, IPNV replication was significantly down-regulated in the vaccinated fish 45 days pi.

© 2014 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-SA license (http://creativecommons.org/licenses/by-nc-sa/3.0/).

1. Introduction

The control of infectious diseases is essential to maintain the levels of productivity in the aquaculture industry. The economic impact of infectious diseases, particularly those of viral aetiology, is a constant worldwide threat in the salmonid fish industry, stimulating research to find efficient methods to minimize such losses. Vaccination is the most effective approach to combat disease in aquaculture, a strategy that is ideal to prevent and avoid the dispersion of infective viruses in fish, particularly in farms where fish are raised under intensive culture conditions. Although different types of viral vaccines have been described for fish, including inactivated, attenuated, synthetic peptides or subunit vaccines [1–3], protection is not always complete. Hence, studies are necessary to produce improved vaccines capable of inducing longer lasting immunity and less stressful methods of administration [4]. Genetic vaccines were first developed for mammals in the 1990s and several designs to protect against rhabdoviruses have been tested in salmonid fish species [5–12]. More recently, other DNA vaccines have been described to combat the infectious pancreatic necrosis virus (IPNV), another viral pathogen of salmonid fish [13–15].

Infectious pancreatic necrosis virus (IPNV) is the type species of the Aquabirnavirus genus, from the *Birnaviridae* family [16]. Virions are non-enveloped and they contain two segments (A, B) of double-stranded RNA. Segment A is the larger of the two (about 3.1 kbp) and it encodes VP2 and VP3, the two major structural proteins of the virus [17,18]. The VP2 protein is the type-specific antigen that can induce the production of neutralizing antibodies that are capable of protecting susceptible fish from viral infection [19–21]. IPNV is one of the main causes of mortality worldwide for juvenile salmonid fish, being especially destructive in salmonid eggs and fingerlings [22]. Fish surviving IPN epizootics develop a persistent viral infection or carrier state, capable of continually transmitting the virus to other susceptible populations of fish, including their own offspring [23–26] (for reviews see Refs. [22,27,28]).

Genetic vaccination for IPNV has only recently been undertaken experimentally, and the initial steps in its development have focused on traditional injection methods [13]. Intramuscular injection of DNA vaccines has been successfully used against viruses such as infectious haematopoietic necrosis virus (IHNV) or viral haemorrhagic septicaemia virus (VHSV). Intraperitoneal injection has also been routinely used for other vaccines, such as recombinant vaccines and multivalent products, and automated systems for

* Corresponding author. Tel.: +34 918373112; fax: +34 915360432.
E-mail address: saraip@cib.csic.es (S.I. Perez-Prieto).

http://dx.doi.org/10.1016/j.fsi.2014.02.003
1050-4648/© 2014 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-SA license (http://creativecommons.org/licenses/by-nc-sa/3.0/).

injection have been developed [3]. However, oral vaccines would be easier to administer and they represent an important alternative to immunize fish against viruses. Nevertheless, there are still few reports of successful vaccine delivery methods other than injection, with delivery in feed having been mostly licensed to prevent bacterial but not viral diseases [4].

We previously described a DNA vaccine derived from the VP2 gene of IPNV inserted into an expression plasmid and encapsulated into alginate microspheres. The oral delivery of the plasmid (diluted in PBS) was performed manually in order to ensure the uniform vaccination of the fish under study. Strong protection was achieved in this way, with around 83% relative survival when challenged 15 and 30 days after vaccine delivery. Indeed, strong expression of IFN and the IFN-induced antiviral Mx protein was recorded 7 and 15 days post-vaccination (pv) [14,29]. However, novel approaches to improve the efficacy of DNA vaccine oral delivery would not only provide interesting data regarding the future mass delivery of these vaccines but also, keys to understand the cellular and mucosal immunity reactions. Oral delivery of DNA vaccines is a process that has been poorly explored, especially against IPNV. Thus, having generated a vaccine that successfully induces appropriate immune protective responses, the next step should be to check if this vaccine can be delivered in feed without losing its beneficial effects due to the severe conditions experienced during gastrointestinal transit.

The goal of the present work was to determine the effectiveness against IPNV of the pcDNA-VP2-encapsulated in alginate and incorporated into fish feed, and the immune responses it induces in rainbow trout. Given that this method appears to produce similar results to those described previously, its potential should be further assessed for industrial application.

2. Materials and methods

2.1. Ethics statement

The experiments described comply with the Guidelines of the European Union Council http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm (Directive 2010/63/EU) for the protection of animals used for scientific purposes and were previously approved by the CSIC Ethics committee.

2.2. Cells and virus

The BF-2 cell line from bluegill fry (*Lepomis macrochirus*, ATCC-CCL 91) was used to isolate and propagate the viruses. The IPNV Sp strain from the ATCC was used in this study (ATCC VR 1318), and all the cells and viruses were cultured as described previously [30]. Briefly, cells were grown at 25 °C in Leibovitz's medium (L15, Gibco, Spain) supplemented with 100 IU mL^{-1} penicillin G, 100 μg mL^{-1} streptomycin, 2 mM L-glutamine and 10% foetal bovine serum (FBS: Gibco, Spain), or with 2% FBS in the maintenance medium (MM). The virus was cultured in L-15 medium and propagated at 20 °C. The virus was titrated in 96-well culture plates (Falcon, Becton–Dickinson) infected with 10-fold serial dilutions and the plates were observed daily for the development of a cytopathic effect (CPE). The infective titres were determined as the 50% infective dose in tissue culture ($TCID_{50}$ mL^{-1}): based on Reed & Muench [31].

2.3. Fish

Rainbow trout (3.5–4 cm and 1.5 g mean size and weight) were purchased from a local spring water farm with no history of viral disease. The fish were kept at the "Centro de Investigaciones Biológicas" (CSIC, Madrid, Spain) under a 12/12 h light/dark regime at 15 °C in 350 L closed re-circulating water tanks (Living Stream, Frigid Units Inc, Ohio). The fish were fed daily with a diet of commercial pellets and they were maintained as described elsewhere [32]. To assess their health, pools of five fish were examined for viruses by standard protocols [33,34], none of the fish lots examined giving positive results. The trout were anaesthetized with buffered tricaine methanesulphonate (MS-222, Sigma) prior to handling and the experiments described comply with the European Union Guidelines (86/609/EU) for the use of laboratory animals.

2.4. Oral vaccination

2.4.1. Vaccine and preparation of fish feed

The pcDNA-VP2 plasmid in *Escherichia coli* (TOP10) was prepared as described previously [14]. Cultures were grown in 10 L of LB broth and the cells were then recovered by centrifugation and frozen at −20 °C. The plasmid was purified from the cells using the QIAGEN plasmid Maxi Kit (Qiagen) according to the manufacturer's instructions. The pcDNA-VP2 and pcDNA plasmids were coated with sodium alginate and these microspheres were prepared as described previously [29]. The feed (T-2.0 Nutra from Trow España S.A, Burgos, Spain) was that recommended for fish that are approximately 3.5 cm long and that weigh 1.5 g, and it was the same as that used at the farm that provided the rainbow trout. This feed contains 54% protein from fish-meal, 18% oil, 1% cellulose, 11.5% ash, 3% calcium, 1.2% sodium and 1.7% phosphorus, as well as several other oligo-elements and anti-oxidants. The particle size ranged from 1.0 to 1.7 mm.

In our previous studies with vaccine–alginate complexes, the fish received daily drops containing 10 μg vaccine. In the present work, the size and weight of the vaccinated trout were similar (3.5 cm, 1.5 g) and since the fish are thought to ingest around 5% of their body weight daily, each fish should receive 0.075 g of feed/day. Thus, the experimental vaccine was prepared in lots of feed for 120 fry trout by placing commercial dry pellets (27 g) into 50 ml Falcon tubes and along with 3.6 mg of pcDNA-VP2-alginate microspheres previously diluted in 15 ml of distilled water. The pellets and microspheres were then mixed gently for a few minutes at room temperature, and the middle-moist feed obtained was lyophilized for 24–48 h and conserved at 4 °C until it was used. In this way, a vaccine concentration of 10 μg per fish and day was achieved.

2.4.2. Fish vaccination

Groups of 25 trout were placed in separate 40 L aquaria maintained at a constant temperature of 15 °C for treatment. The first group of fish was vaccinated by providing with vaccine impregnated food pellets at 5% of body weight for three consecutive days (10 μg pcDNA-VP2/fish/day). The second group of rainbow trout was fed with pellets mixed with the empty pcDNA plasmid, serving as the plasmid control, and the third group of fish received the commercial pellets and was considered as the untreated mock vaccinated fish control. An additional group of fish were vaccinated individually with a pcDNA-VP2 alginate microspheres solution in water, and used as a positive control in the light of the results obtained with this method elsewhere [29].

2.4.3. Tissue distribution and time-course of pcDNA-VP2 expression

At 1, 3, 5 and 15 days pv (after the last feed), 3 trout from each group were sacrificed with an overdose of MS-222, and the kidney, spleen, liver, gut and gill tissue was removed aseptically. Total RNA was isolated using the TRIzol reagent (Invitrogen, Spain), according to the manufacturer's instructions, and treated with DNase I to remove any trace genomic DNA that might interfere with the PCR reactions. Equal amounts of RNA were primed with oligo(dT) and

Download English Version:

https://daneshyari.com/en/article/10972020

Download Persian Version:

https://daneshyari.com/article/10972020

Daneshyari.com