Vaccine 35 (2017) 2420–2426

Contents lists available at ScienceDirect

Vaccine

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

A effective DNA vaccine against diverse genotype J infectious hematopoietic necrosis virus strains prevalent in China

Liming Xu [a], Jingzhuang Zhao [a], Miao Liu [a], Gael Kurath [b], Guangming Ren [a], Scott E. Lapatra [c], Jiasheng Yin [a], Hongbai Liu [a], Jian Feng [d], Tongyan Lu [a,*]

[a] Heilongjiang River Fishery Research Institute Chinese Academy of Fishery Sciences, Harbin 150070, PR China
[b] Western Fisheries Research Center, USGS Biological Resources Division, Seattle, WA 98115, USA
[c] Clear Springs Foods Inc., Research Division, Buhl, ID, USA
[d] Benxi Agrimarine Company Limited, Benxi 117000, PR China

ARTICLE INFO

Article history:
Received 18 January 2017
Received in revised form 6 March 2017
Accepted 11 March 2017
Available online 23 March 2017

Keywords:
Cross-protection
DNA vaccine
Intragenogroup challenge
Infectious hematopoietic necrosis virus
Rhabdovirus
Genogroup J

ABSTRACT

Infectious hematopoietic necrosis virus (IHNV) is the most important pathogen threatening the aquaculture of salmonid fish in China. In this study, a DNA vaccine, designated pIHNch-G, was constructed with the glycoprotein (G) gene of a Chinese IHNV isolate SD-12 (also called Sn1203) of genotype J. The minimal dose of vaccine required, the expression of the Mx-1 gene in the muscle (vaccine delivery site) and anterior kidney, and the titers of the neutralizing antibodies produced were used to evaluate the vaccine efficacy. To assess the potential utility of the vaccine in controlling IHNV throughout China, the cross protective efficacy of the vaccine was determined by challenging fish with a broad range of IHNV strains from different geographic locations in China. A single 100 ng dose of the vaccine conferred almost full protection to rainbow trout fry (3 g) against waterborne or intraperitoneal injection challenge with IHNV strain SD-12 as early as 4 days post-vaccination (d.p.v.), and significant protection was still observed at 180 d.p.v. Intragenogroup challenges showed that the DNA vaccine provided similar protection to the fish against all the Chinese IHNV isolates tested, suggesting that the vaccine can be widely used in China. Mx-1 gene expression was significantly upregulated in the muscle tissue (vaccine delivery site) and anterior kidney in the vaccinated rainbow trout at both 4 and 7 d.p.v. Similar levels of neutralizing antibodies were determined with each of the Chinese IHNV strains at 60 and 180 d.p.v. This DNA vaccine should play an important role in the control of IHN in China.

1. Introduction

Infectious hematopoietic necrosis virus (IHNV) is a typical virus of the genus *Novirhabdovirus* and a major pathogen of salmon and trout [1]. Depending upon the differences on host species, virus strains, and fish farming environment, Epidemics of infectious hematopoietic necrosis (IHN) can cause mortality at rates exceeding 90% in some cases, depending upon the host species, viral strain, and fish-farming environment [2]. To reduce the economic losses caused by this pathogen, various candidate IHNV vaccines have been developed [3,4], including attenuated vaccines [5–10], killed virus [11], and vaccines based on recombinant DNA technology. However, traditional vaccines have not provided the desired protection to rainbow trout [12,13]. The first DNA vaccine developed against IHNV stimulated a strong immune response in rainbow trout fry with a single dose of 10 μg [14]. Another IHNV DNA vaccine based on the G gene of IHNV strain WRAC conferred almost full protection at a lower dose [2,12,15–17], and the vaccine, designated pIHNw-G, was very stable and effective under various conditions, including in various fish host species and life stages, during challenges with diverse strains, and when delivered with various strategies [12,15,17–19].

According to previous phylogenetic studies, the worldwide IHNV strains can be divided into five genogroups U, M, L [20–25], J [26,27] and E [28,29]. All Chinese IHNV isolates cluster in the J Nagano sub genogroup within genotype J, together with some Japanese IHNV isolates [30]. Although they all belong to genogroup J, the IHNV isolates that have evolved in Chinese salmon environments formed a separate branch [30]. Previous IHNV DNA vaccines were constructed with the G genes of the IHNV genogroup M and U

* Corresponding author.
E-mail addresses: 279463437@qq.com (L. Xu), 422842454@qq.com (J. Zhao), 446048847@qq.com (M. Liu), gkurath@usgs.gov (G. Kurath), 171066690@qq.com (G. Ren), scott.lapatra@clearsprings.com (S.E. Lapatra), xwsc20@tom.com (J. Yin), 112893129@qq.com (H. Liu), jfeng666@126.com (J. Feng), lutongyan@hotmail.com, lutongyan@hrfri.ac.cn (T. Lu).

http://dx.doi.org/10.1016/j.vaccine.2017.03.047
0264-410X/

strains [14,16], and Corbeil et al. [12] demonstrated that the pIHNw-G vaccine protects fish against a broad range of viral strains from different geographic locations, including strains from France and Japan. However, Chinese IHNV strains have not yet been tested. In this study, we constructed an IHNV DNA vaccine based on the G gene of the J genotype IHNV- SD12 strain (also called Sn1203) isolated from rainbow trout [31]. The protection efficacy of the DNA vaccine was determined with intragenogroup challenges using nine field IHNV isolates from different Chinese provinces, which together include almost all the salmon and trout aquaculture districts in China. To our knowledge this is the first study to construct an IHNV DNA vaccine using the G gene from a genogroup J IHNV strain. The protection efficacy of the DNA vaccine was assessed by intragengroup challenges with IHNV field strains recently prevalent in China. This DNA vaccine should play an important role in the control of IHN in China.

2. Material and methods

2.1. Ethics statement

In this study, all animal experiments were performed under the Guidelines of European Union Council Directive 2010/63/EU (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm) for the protection of animals used for scientific purposes.

2.2. Materials

Nine Chinese IHNV strains isolated from different geographic locations in China in 2012–2015 were from laboratory stocks: SD-12 (also called Sn1203), HLJ-15, LN-15, BJ-15, GS-15, XJ-14, QH-15, SC-15, and YN-15. The locations and detailed information on these IHNV strains were given in Fig. 1 and Table 1 in the Supplementary Materials. *Epithelioma papulosum cyprini* (EPC) cells were kindly provided by Professor Lingbing Zeng of the Changjiang River Fisheries Research Institute, Chinese Academy of Fishery Sciences, China.

2.3. DNA vaccine

The DNA vaccine, designated pIHNch-G, was constructed by cloning the G gene of SD-12 into plasmid pcDNA3.1. The vaccine pIHNch-G and plasmid pcDNA3.1 were propagated in *Escherichia coli* strain DH5a and prepared with an EndoFree plasmid extract kit (Tiangen, Shanghai, China). The expression of the G gene from the DNA vaccine in fish cells was confirmed by transfecting the fish EPC cell line with pIHNch-G, and an immunofluorescence antibody test (IFAT) as described in a previous study [32].

2.4. DNA vaccination

Specific-pathogen-free rainbow trout (mean weight, 3 g; Heilongjiang River Fishery Research Institute, Harbin, China) were anaesthetized by immersion in tricaine methane sulfonate (MS-222; Sigma, USA) and the DNA vaccine was delivered by intramuscular (i.m.) injection at the base of the dorsal fin. To determine the optimal dose of the DNA vaccine, 0.1, 1.0, 5.0 and 10.0 μg was injected in 50 μl of phosphate-buffered saline (PBS). The control fish were injected with the vector alone, PBS alone, or left unhandled, as specified in the text. Each treatment group was placed in a 50 L circulating water tank (15 °C) and fed a dry pelleted diet *ad libitum*.

2.5. Challenges of rainbow trout with SD-12

The rainbow trout was challenged by immersion or by intraperitoneal (i.p.) injection, depending on the size of the fish. In the immersion challenge experiment, duplicate groups of 50 fish each (mean weight $\leq$ 5 g) were exposed to waterborne SD-12 for 60 min with aeration. The concentration of SD-12 was 10^5 plaque-forming units (PFU)/ml, and the volume of water was 10× the total weight of the fish (g). In the i.p. injection challenge, each fish from duplicate groups of 50 fish (mean weight over 5 g) was anesthetized and injected i.p. with 10^2 PFU SD-12 in 50 μl of PBS. Mock infections were performed by replacing the viral suspension with PBS. The experimental groups were maintained separately in 50 L circulating tanks (15 °C) and fed a dry pelleted diet *ad libitum*. The cumulative percentage mortality (CPM) was monitored for 21 days. The relative percentage survival (RPS) was then calculated with the formula RPS = [1 − (% mortality of fish given vaccine/% mortality of fish given pcDNA3.1)] × 100 [33]. In the DNA vaccine dose experiments, the fish were challenged by immersion at 21 d.p.v. To determine the early protection and specific protection conferred by the DNA vaccine, rainbow trout were immersion challenged with SD-12 at 1, 2, 4, or 7 d.p.v. or with i.p. injection at 28, 120, or 180 d.p.v.

2.6. Intragenogroup challenge experiments

To determine the cross protection afforded by the DNA vaccine against diverse Chinese IHNV strains, duplicate groups of 50 fish were immersion challenged at 4 d.p.v. or challenged with i. p. injection at 60 or 180 d.p.v. Each IHNV strain (Table 1 in the Supplementary Materials) was used as a challenge strain, and the challenge dose was the same as that used for of SD-12 (Section 2.5).

2.7. Mx-1 gene expression assay

RNA from the muscle tissues (at the injection site) and anterior kidneys of the immunized fish (n = 5) was prepared with routine procedures. Quantitative reverse transcription-PCR (qRT-PCR) was performed with the One Step SYBR PrimeScript Plus RT-PCR Kit (Perfect Real Time) (Takara, Dalian, China, cat. no. RR096A). The housekeeping gene, acidic ribosomal phosphoprotein P0 (ARP) [34] was used as the reference gene to normalize the expression level of the Mx-1 gene. The fold-change in Mx-1 gene expression was calculated relative to that in pcDNA3.1 in the PBS-mock-vaccinated control group.

2.8. Neutralizing antibody titers

At 60 and 180 d.p.v., blood samples (n = 10) from the trout vaccinated with pIHNch-G or empty pcDNA3.1 (without viral challenge) were collected by caudal transection, and sera were prepared with a routine procedure. The IHNV-neutralizing antibody (NAb) titer of each serum was determined with a complement-dependent neutralization assay. Titers of $\geq$20 were considered positive, and a titer of <20 was negative [35].

2.9. Statistical analysis

Analysis of variance (ANOVA) was used to determine the differences in gene expression level replicates. Student's t test was also used to compare some paired samples. Values of $P < 0.05$ were deemed statistically significant.