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Protective immunity and lack of histopathological damage two years after DNA vaccination against infectious hematopoietic necrosis virus in trout

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Abstract

The DNA vaccine pIHNw-G encodes the glycoprotein of the fish rhabdovirus infectious hematopoietic necrosis virus (IHNV). Vaccine performance in rainbow trout was measured 3, 6, 13, 24, and 25 months after vaccination. At three months all fish vaccinated with 0.1 μg pIHNw-G had detectable neutralizing antibody (NAb) and they were completely protected from lethal IHNV challenge with a relative percent survival (RPS) of 100% compared to control fish. Viral challenges at 6, 13, 24, and 25 months post-vaccination showed protection with RPS values of 47–69%, while NAb seroprevalence declined to undetectable levels. Passive transfer experiments with sera from fish after two years post-vaccination were inconsistent but significant protection was observed in some cases. The long-term duration of protection observed here defined a third temporal phase in the immune response to IHNV DNA vaccination, characterized by reduced but significant levels of protection, and decline or absence of detectable NAb titers. Examination of multiple tissues showed an absence of detectable long-term histopathological damage due to DNA vaccination.

Keywords: DNA vaccination; Long-term protection; Fish DNA vaccine; DNA vaccine safety

1. Introduction

The goal of this study was to assess the long-term duration of protection provided to rainbow trout by a DNA vaccine against infectious hematopoietic necrosis virus (IHNV). IHNV is an acute rhabdoviral pathogen of many salmon and trout species [1,2]. It is endemic to western North America where it causes epidemics in both wild and cultured fish, and it has also become established in European and Asian aquaculture. There is currently no commercially available efficacious vaccine against IHNV despite numerous efforts over the last 25 years [2–4]. Therefore, an efficacious IHNV vaccine is actively being sought for use in trout farms and Atlantic salmon seapen aquaculture as well as for natural resource salmonid hatcheries.

In 1996 the first report of genetic immunization of fish described an IHNV DNA vaccine that provided strong protective immunity to juvenile rainbow trout (*Oncorhynchus mykiss*) challenged six weeks post-vaccination [5]. This vaccine contained the viral gene for the antigenic surface glycoprotein (G protein) of the IHNV reference strain RB1. Another IHNV DNA vaccine was subsequently developed containing the G protein gene of the second IHNV reference strain, WRAC, that originated from rainbow trout in

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an Idaho trout farm [6]. This vaccine, designated pIHNw-G, is effective at extremely low doses; single intramuscular (i.m.) injections of juvenile rainbow trout with 0.1 or 1.0 μg DNA typically provide protection with 80–100% relative percent survival (RPS) compared with mock-vaccinated control groups [6–10]. The pIHNw-G vaccine has been shown in numerous studies to be highly efficacious under a wide variety of conditions including various fish host species and life stages, challenge with diverse IHNV strains, and delivery by intramuscular (i.m.) injection or gene gun [7,8,10–12]. In a parallel host–viral system, similar high efficacy has been demonstrated for a DNA vaccine against the related rhabdovirus, viral hemorrhagic septicemia virus (VHSV) [13–15], which causes epidemics in rainbow trout in Europe. The exceptionally high efficacy of the DNA vaccines against these two fish rhabdoviruses has resulted in their establishment as model systems for study of DNA vaccines in fish [16–18].

In most of the efficacy studies with these fish DNA vaccines the standard length of time between vaccination and viral challenge was four–six weeks. However, both the IHNV and VHSV DNA vaccines have been shown to elicit an extremely rapid onset of innate immunity that is protective by four–seven days post-vaccination and is cross-protective against several fish virus species [9,19]. This early protective phase is followed by a second phase of very strong, specific protection that correlates with the development of detectable neutralizing antibodies [9,12,16]. With regard to the long-term duration of protection provided by the fish rhabdovirus DNA vaccines, it has been reported that pIHNw-G elicited significant protection against viral challenge 80 days post-vaccination [6], and the VHSV DNA vaccine was protective at six and nine months post-vaccination [13,20]. The current study was undertaken to assess the ability of the IHNV DNA vaccine to protect fish at timepoints out to two years post-vaccination, and to examine immune responses and potential histopathological consequences during this period.

2. Materials and methods

2.1. Fish vaccination and maintenance

Two cohorts of research grade juvenile rainbow trout (*Oncorhynchus mykiss*) were obtained from Clear Springs Foods Inc. approximately one year apart. Fry were maintained in sand-filtered and UV treated lake water at 12 °C and fed a semi-moist pelleted diet (Bio-Oregon, Warrenton, OR) at 1.5% body weight per day. Construction and preparation of the pIHNw-G DNA vaccine and the pLuc negative control vaccine have been described previously [8]. Fish from cohort 1 were used for viral challenge experiments, serological analyses, histology, and passive transfer experiments 1 and 2. For these studies two groups of 500 juvenile rainbow trout from cohort 1 (mean weight 2.5 g) were anaesthetized by immersion in 100 μg/ml tricaine methane sulfonate (MS-222; Argent Chemical Laboratories, Redmond, WA) and vaccinated by i.m. injection [8] with 0.1 μg of either pIHNw-G or pLuc in a total volume of 50 μl phosphate buffered saline (PBS). A group of 200 fish was left unhandled, and all fish were maintained at 12 °C for the entire study period.

Fish cohort 2 was used only for collecting sera 26 months post-vaccination that was used in passive transfer experiments 3–6. For this four groups of 25 cohort 2 fish (mean weight 1.1 g) were vaccinated as above with either 0.1 or 1.0 μg of pIHNw-G, or 0.1 or 1.0 μg pLuc.

2.2. Viral challenge experiments

IHNV strain 220–90, which is virulent in rainbow trout, was used for challenge studies. Virus was propagated in the *epithelioma papulosum cyprini* (EPC) cell line in supplemented minimal essential medium (MEM) as previously described [8], and quantified by plaque assay [21]. All challenges were done by intraperitoneal (i.p.) injection because of the known decrease in susceptibility of rainbow trout to IHNV immersion challenge with increased age and size [22]. Approximately one month prior to each challenge timepoint a pilot test for viral challenge dose was conducted in which groups of three–four unhandled fish from cohort 1 were injected i.p. with 200 μl MEM containing IHNV doses ranging from 10^3 to 10^8 plaque forming units (pfu) per fish. The lowest virus dose that resulted in 67–100% mortality was selected for use in challenge experiments (Table 1).

At 3, 6, 13, and 24 months post-vaccination three subgroups of 25 pIHNw-G vaccinated fish and three subgroups of 25 pLuc vaccinated fish were transferred to individual tanks for a viral challenge experiment. At 25 months post-vaccination a challenge experiment was done with three subgroups of 18 fish per treatment. For each experiment duplicate groups of pIHNw-G vaccinated fish were i.p. injected with IHNV as described above, and the third group was injected with 200 μl MEM without virus. Similarly, two groups of pLuc vaccinated fish were challenged with IHNV, and the third group was mock-challenged. Fish were monitored for mortality daily for 30 d, and a minimum of 20% of the dead fish in each experiment were titered for virus by plaque assay [21]. Vaccine efficacy was determined by comparing the average cumulative percent mortality (CPM) and relative percent survival (RPS, calculated as specified in Table 1) [23] between pIHNw-G and pLuc treatment groups. RPS values are considered most valid when CPM in negative controls is ≥60 [24]. Survival curves (% mortality) were estimated using the Kaplan–Meier method, and a log-rank test was used to compare the survival curves (SPSS 11.5 for Windows, SPSS Inc., Chicago, IL), both between replicate tanks within groups, and between groups. Data with $p < 0.05$ were considered to show a significant difference. Analysis of replicate tanks within groups showed no significant difference at any timepoint except for the replicate pLuc-vaccinated groups in the 24-month timepoint. Therefore, replicates were pooled for treatment groups within each of the 3, 6, 13, and 25-