Fish & Shellfish Immunology 34 (2013) 387–392

Contents lists available at SciVerse ScienceDirect

Fish & Shellfish Immunology

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

Short communication

Response to vaccination of Atlantic cod (*Gadus morhua* L.) progenies from families with different estimated family breeding values for vibriosis resistance

Helene Mikkelsen*, Marit Seppola

Nofima (Norwegian Institute of Food, Fishery and Aquaculture), P.O. Box 6122, N-9291 Tromsø, Norway

ARTICLE INFO

Article history:
Received 28 February 2012
Received in revised form
5 October 2012
Accepted 7 October 2012
Available online 23 October 2012

Keywords:
Atlantic cod
Vibriosis
Vaccination
Resistance
Innate immune response

ABSTRACT

The purpose of the study was to elucidate whether responses to vibriosis vaccination and gene expressions in parts of the innate immune system were different in families of Atlantic cod (*Gadus morhua*). The fish were progenies of families with differences in estimated breeding values (EBV) for vibriosis resistance. Families of coastal cod (CC) and northeast Arctic cod (AC) responded well to vaccination with a relative percent survival of 72–95. No correlation between response to vaccination and vibriosis resistance were found ($p = 0.146$). The AC family with medium low (M) resistance had significant ($p \leq 0.019$) lowest mortality among all the unvaccinated fish but the CC-M family. Further, when comparing the vaccinated fish the AC family with very high (VH) resistance had significant ($p \leq 0.004$) higher mortality than all except the CC-VL and CC-H families.

Parts of the innate immune response were studied by measuring the gene expression of innate immune genes 2 and 4 days post dip vaccination. Vaccinated fish from two families had a weak but significant higher innate immune response compared to control fish of the same family. In vaccinated fish, the gene expression of interleukin (IL) 1b, IL-10, IL-12p40 and hepcidin were significant up-regulated. While, no measureable activations of interferon gamma (IFNγ), IL-8, cathelicidin, LBP/BPI and G-type lysozyme were found.

1. Introduction

The Norwegian cod breeding program is based on brood-stocks of Atlantic cod (*Gadus morhua* L.) subpopulation of coastal and northeast Arctic cod. Resistance to vibriosis has been one of the breeding parameters as classical vibriosis caused by *Vibrio anguillarum* is common in Atlantic cod aquaculture even in vaccinated fish [1,2]. The heritability of vibriosis resistance has been estimated to be moderate (0.16 ± 0.04) calculated by the threshold model [3]. Previous challenge studies with *V. anguillarum* O2b revealed that the subpopulation of coastal cod were significantly more resistant than northeast Arctic cod [4,5]. Experimental dip vaccination against vibriosis of Atlantic cod resulted in high protection but in cod aquaculture the protection has been variable [6,7]. This may be due to different practise among farmers in following the recommended vaccination strategies and/or multiple stress factors present in aquaculture, but also by differences in vibriosis resistance and response to vaccination among cod families. Studies of other fish species regarding diseases susceptibility/resistance among genetically different families have mainly focused on identifying immune parameters that could explain the observed differences. Previous studies of cod [6] and rainbow trout (*Onchorhynchus mykiss* W.) [8] have only detected a weak or absent innate and humoral immune response following dip vaccination, in spite of high protection in fish. A clear correlation between natural resistance against disease and immunological responses has not been found, but some indications have been proposed [9–13]. Furunculosis resistant Atlantic salmon (*Salmo salar* L.) had higher immune response such as activity of serum complement and non-alpha(2)m antiprotease [9], complement activity and increased expression of pro-inflammatory genes, compared to sensitive fish [13]. In goldfish (*Carassius auratus* L.) a correlation between high amount of natural antibodies and resistance to *Aeromonas salmonicida* infection have been indicated [14]. Difference in susceptibility to disease and sensitivity to stress has been shown to have an impact on the immune response. Genetically different common carp (*Cyprinus carpio* L.) challenged with *Aeromonas hydrophila* showed that resistant carp had higher production of specific antibodies, phagocytic and plasma lysozyme activity [15]. Common carp selected for low stress response had both higher specific antibody production and survival rate than the high stress responders after vaccination with *A. salmonicida*/*A. hydrophila* [16]. In this study, we have compared the response to dip vaccination in cod families that were

* Corresponding author. Tel.: +47 77 62 90 00; fax: +47 77 62 91 00.
E-mail address: helene.mikkelsen@nofima.no (H. Mikkelsen).

1050-4648/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fsi.2012.10.010

progenies of parents with different estimated breeding values for vibriosis resistance ranging from very high to very low. The vaccination response was measured as protection following bath challenge with homologous strain. In addition, activation of parts of the innate immune responses was studied.

2. Materials and methods

2.1. Bacteria

The *V. anguillarum* serotype O2b isolate 4299 used for vaccination and challenge in this study has been used in previous challenge experiments with cod from the Norwegian Cod Breeding program (NCBP: Tromsø, Norway) [4,5] and in several vaccination trials [6,7,17–20]. Growth conditions and determination of colony forming units (cfu) of challenge doses and re-isolation of *V. anguillarum* from moribund and dead fish, were done as previously described [6].

2.2. Fish

Atlantic cod juveniles had genetic background from either coastal cod (CC) or northeast Arctic cod (AC), respectively (Table 1). It was 7 full- and 3 half-sib families of the year-class 2009 from the F2 generation of selected fish of year-class 2006 from NCBP. The families were selected based on the estimated family breeding value (EBV) for resistance to vibriosis in the parental fish and were ranked from very low (VL), low (L), medium low (M), high (H) to very high (VH). These abbreviations will be used from here on. Resistance were defined as the number of days from challenge to death or until end of challenge-test (censored observation) using a multivariate linear model [5] and a threshold model [3]. The standardized scale of family EBV is calculated as $100 + 10^{(\text{Family EBV}-\text{Mean of Family EBV})}$/Standard Deviation. The CC-H were half-siblings with CC-L1 (same dams) and AC-M (same sires), and the AC-VL was half-siblings with AC-H (same dams). The fish were transported (at approx. 1.6 g) to the Aquaculture Research Station (Tromsø, Norway) for grow-out in seawater of 3.4% salinity at 10 °C, 24 h light and fed with commercial feed (BioMar, Norway). The rates of water inflow were adjusted to an oxygen saturation of 90–100 % in the outlet water. The fish were reported to be healthy without any history of diseases and the experiment was approved by the National Animal Research authority in Norway.

2.3. Vaccines and vaccination experiment

The experimental dip vaccine produced by PHARMAQ AS (Norway) contained bacterin of *V. anguillarum* serotype O2b isolate 4299. Cod (approx. 2.5 g) were dip vaccinated by immersion for 30 s in diluted vaccine (1:10 in seawater), according to the manufacturer's instruction. One control group per family was mock-vaccinated, by dipping in seawater without vaccine. The fish, 10 vaccinated and 10 control groups, were distributed in 20 parallel circular centrally drained, fibreglass tanks (100 l) with approx. 100–125 fish in each tank (density <20 kg dm^3). Fish for pre-challenge ($n = 72$) were left untreated and kept in a separate tank. One week prior to challenge the fish were anaesthetised with Metacainum (Norsk Medisinaldepot, Norway) (70 mg l^{-1}) and marked at the operculum Visible Implant Fluorescent Elastomer (Northwest Marine Technology Inc. US) before distributed in 4×500 l tanks (2 tanks with CC and AC families, respectively) with 80 fish from each family (40 vaccinated and 40 controls) in each tank (Table 1).

2.4. Experimental challenges

A prechallenge experiment was performed to determine the bath challenge doses resulting in 60–80% mortality in unvaccinated cod as described previously [6]. The fish were distributed in four 30 l tanks ($n = 18$) and acclimatised to 10 °C one week prior to bath challenge. In each tank the fish was exposed to the virulent bacteria for 60 min in a reduced water volume with oxygenation. Then outlet and inlet was opened and water flow was restored to normal. In addition, one control group in the prechallenge experiment was treated as the challenged fish but was not exposed to bacteria. Mortality was recorded daily and dead fish were removed. Mortality showed a dose-depended result and a dose of 6.5×10^6, 6.5×10^5 and 6.5×10^4 cfu ml^{-1} resulted in 83, 44 and 18% cumulative mortality, respectively. No mortality was recorded in control fish. A dose of approx.1×10^6 cfu ml^{-1} was chosen for the challenge of vaccinated and control fish.

Vaccine responses were determined 7 weeks post vaccination by bath challenges in parallel tanks (Fig. 1). Two days before challenge the pre-sorted vaccinated and unvaccinated fish from the different families were transferred to 4×500 l tanks at the fish health unit of The Aquaculture station. Mortality was recorded daily and dead fish were removed. The cause of death was verified by isolation of bacteria in monoculture from the head kidney on blood agar supplemented with 1.5% NaCl, and further confirmed by agglutination with specific MonoVa antibodies (BioNor AS, Norway).

2.5. Innate immune gene expression

Head kidney and spleen ($n = 6$) were collected 0, 2 and 4 days post vaccination from 6 of 10 families (4 CC and 2 AC) in RNA later (Ambion, Applied Biosystems, US). Homogenisation of pooled samples of head kidney and spleen was performed using the MagNA Lyser Green Beads and the MagNa Lyser Instrument (Roche

Table 1
Vibriosis resistance, background, marking and estimated breeding values (EBV) of parents of coastal (CC) and northeast Arctic (AC) cod families used in the study.

Groups	Vibriosis resistance	Background	Family EBV	Stand[a] EBV	Marking Con[b]	Vac	Challenge tank
CC-VL	Very low	¾ CC + ¼ AC	−1.2629	−1.81	Red R[c]	Red L	1 and 2
CC-L1	Low	¾ CC + ¼ AC	−1.2269	−1.44	Green R	Green L	
CC-L2	Low	¾ CC + ¼ AC	−1.2078	−1.24	Orange R	Orange L	
CC-M	Medium low	¾ CC + ¼ AC	−1.1495	−0.64	Pink R	Pink L	
CC-H	High	¾ CC + ¼ AC	−0.9829	1.06	Read H	Green H	
AC-VL	Very low	¾ AC + ¼ CC	−1.3259	−2.45	Red R	Red L	3 and 4
AC-L	Low	¾ AC + ¼ CC	−1.1826	−0.98	Green R	Green L	
AC-M	Medium low	AC	−1.1289	−0.43	Orange R	Orange L	
AC-H	High	AC	−1.0074	0.81	Pink R	Pink L	
AC-VH	Very high	AC	−0.8645	2.28	Read H	Green H	

[a] Stand: Standardized scale = $100 + 10^{(\text{family EBV\_Mean of Family EBV})}$/Standard Deviation.
[b] Con: control (mock-vaccinated), Vac: dip vaccinated.
[c] Right (R) or left (L) hand side of the fish operculum, H: head of the fish.