Fish & Shellfish Immunology 26 (2009) 385–395

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Ontogeny of lymphoid organs and development of IgM-bearing cells in Atlantic halibut (*Hippoglossus hippoglossus* L.)

Sonal Patel [a,b,*], Elin Sørhus [a], Ingrid Uglenes Fiksdal [a], Per Gunnar Espedal [c], Øivind Bergh [a,c], Odd Magne Rødseth [a,1], H. Craig Morton [a], Audun Helge Nerland [a,b,2]

[a] Institute of Marine Research, Nordnesgaten 50, N-5817 Bergen, Norway
[b] Department of Molecular Biology, University of Bergen, Thormøhlensgate 55, N-5008 Bergen, Norway
[c] Institute of Biology, University of Bergen, Thormøhlensgate 55, N-5008 Bergen, Norway

ARTICLE INFO

Article history:
Received 14 October 2008
Received in revised form
13 November 2008
Accepted 25 November 2008
Available online 10 December 2008

Keywords:
Larvae
Juvenile
Real time RT-PCR
Gene expression
In situ hybridisation
IHC
Teleost

ABSTRACT

In teleost fish, the head kidney, thymus, and spleen are generally regarded as important immune organs. In this study, the ontogeny of these organs was studied in Atlantic halibut (*Hippoglossus hippoglossus*), larvae at various stages of development. We observed that the kidney was present at hatching, the thymus at 33 days post hatch (dph), while the spleen was the last organ to be detected at 49 dph. All three lymphoid organs were morphologically well developed during late metamorphic stages. Real time RT-PCR analysis showed that IgM mRNA expression could be observed at 66 dph and later, which correlates well with *in situ* hybridisation data showing that a few IgM positive cells could be detected in the anterior kidney and spleen from 66 dph. Our data also showed that the highest levels of IgM mRNA could be detected in halibut spleen. Immunostaining using a monoclonal antibody against halibut IgM detected IgM positive cells at 94 dph in both the head kidney and the spleen, which is much later than the IgM mRNA. Numerous cells expressing both IgM mRNA and protein could be detected in the spleen and anterior kidney and also to some extent in thymus specimens from adult halibut.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Atlantic halibut larvae are primitive at hatching, and have a prolonged yolk sac stage. Halibut ontogeny is described [1,2] and information on thymus development and function during some of the important developmental stages has been reported (reviewed by ref. [3]). However, very little information regarding ontogeny of the lymphoid organs and maturation of the immune system, especially during the late larval and juvenile stages, is available. Marine larvae hatch in an environment where they are exposed to numerous pathogens. In nature, they feed on algae and zooplankton during the early life stages. In halibut aquaculture, live feed such as *Artemia* has mostly replaced natural zooplankton. Live feed cultures have high densities of organic matter, and may subject the larvae to a significant bacterial load that has been associated with high rates of mortality (reviewed by refs. [4,5]). It is believed that larvae/fry during early life stages are protected by maternally transferred components like lectins and antibodies, and are dependent on their innate defence mechanisms that are present during early egg stages ([6,7], reviewed by refs. [8–12]). However, these mechanisms do not always suffice during an encounter with pathogens. This emphasises the need to establish adequate prophylactic counter measures like vaccination and use of probiotics. Development of successful vaccines depends mainly on the ability to activate the specific immune system, and synthesise antibodies and memory cells. However, immune stimulation before the fish is able to mount an effective immune response can induce tolerance resulting in the lack of response to later stimulation [13–15].

Extensive numbers of studies have been reported on organogenesis of commercially important freshwater species like salmon, rainbow trout, and carp [16,17]; and for marine species like turbot, Japanese flounder, Atlantic cod, Senegalese sole and sea bass (reviewed by ref. [18]). Lymphoid organs such as the head kidney, thymus, and spleen are usually formed during larval development [19,20]. The ontogeny of these organs and the appearance of IgM-bearing cells constitute key events in the maturation of the adaptive immune system.

* Corresponding author. Institute of Marine Research, Fish Health, Nordnesgaten 50, N-5817 Bergen, Norway. Tel.: +47 5523 5345; fax: +47 5523 8531.
E-mail address: sonal.patel@imr.no (S. Patel).
[1] Present address: Aqua Gen AS, Havnegata 9, Trondheim, Norway.
[2] Present address: The Gade Institute – Oral Microbiology, University of Bergen, N-5021 Bergen, Norway.

1050-4648/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fsi.2008.11.018

The characterisation of the origin and the time of appearance of B- and T- lymphocytes enhance the understanding of the ability of a particular species to generate humoral and cell mediated immune responses. Although there are reports describing organogenesis in different marine species (reviewed by ref. [18]) and on the ontogenesis of important immune related organs and cells in many fish species [16,19,20,21,23–37], very little information on halibut is available.

Here we describe the ontogeny of the kidney, thymus, and spleen during larval development in Atlantic halibut. The ontogeny of the lymphoid organs was studied morphologically at different developmental stages from eggs to juvenile. IgM positive cells were detected by immunostaining with an anti-halibut IgM monoclonal antibody. In addition, real time RT-PCR and *in situ* hybridisation analysis was performed in order to correlate the expression of IgM mRNA and IgM protein at various stages during the development process. The information will facilitate development of prophylactic measures such as vaccination and non-specific immunostimulation in halibut.

2. Methods and materials

In this study samples were collected from the day of hatching, from the larval and juvenile stages, and from adult fish that had reached a weight of approximately 150 g. For practical reasons, some larval and adult samples were taken from different fish cohorts. Samples obtained from cohort 1 up to 41 days post hatch (dph), were used for morphological studies while samples obtained from cohorts 2 and 3 were used for morphological studies, immunohistochemistry (IHC), *in situ* hybridisation, and real time RT-PCR from 49 dph and onwards.

2.1. Fish

2.1.1. Cohort 1

Ten days post fertilisation (dpf), halibut eggs were transferred to six-well plates (Nunc), one egg per well containing 10 ml of sterile sea water (SSW) with a salinity of 25 ppt [38]. The eggs were inspected daily and the day the eggs hatched the chorions removed to decrease the risk of bacterial contamination. Larvae from multiwell plates were sampled at the following time points: 1, 11, 18, 21, 27, 33, 37, 41, and 49 dph [6, 66, 108, 126, 162, 198, 222, 246 and 294 day degrees post hatch (ddph) respectively]. The larvae were not fed and maintained in multiwell plates in an air conditioned room at 6 °C until the end of sampling.

2.1.2. Cohort 2

Eggs from one female from the halibut broodstock maintained at Austevoll Aquaculture Research Station were stripped, and fertilised with sperm from a single male. Eggs and larval stages up to 45 dph were maintained at 6 °C, and larval stages from 45 dph and onwards at 12 °C as described [39]. The larvae were fed with enriched *Artemia* until weaning to dry feed at approximately 120 dph. All sampling at 49, 52, 59, 66, 73, 80, 87, 94, 102, 108, 115, 122, 129, 144, and 159 dph (318, 354, 438, 522, 606, 690, 774, 858, 954, 1026, 1110, 1194, 1278, 1458, and 1638 ddph, respectively) was carried out from tanks.

To avoid disturbing larvae during the critical yolk sac phase and to ease sampling during the rearing period in silo, some fertilised eggs were also transferred to six-well plates at 10 dpf with 10 ml of sterile sea water (SSW) with a salinity of 25 ppt as described earlier. Approximately eight eggs were transferred to each well, and the eggs were inspected daily. The day after hatching, two larvae from each well were transferred to new plates to reduce the risk of bacterial contamination. Larvae from multiwell plates were sampled at the following dph: 1, 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, and 45 (6, 18, 42, 60, 84, 102, 126, 144, 168, 186, 210, 228 and 270 ddph, respectively).

2.1.3. Cohort 3

In addition to the experiments mentioned above, Atlantic halibut weighing 70–150 g were obtained from Austevoll aquaculture research station, Norway. For sampling of various organs, fish were euthanised with an overdose of benzocain. To study differential expression of IgM using real time RT-PCR analysis, samples of thymus, spleen, skin, heart, anterior and posterior kidney, pectoral fins, gills, brain, liver, anterior and posterior gut were obtained from four healthy fish. All samples taken for PCR based analysis were snap-frozen in liquid nitrogen immediately after dissection and stored at −80 °C until needed. From the same cohort, samples were taken from spleen, thymus, and anterior kidney from one fish for immunohistochemistry and *in situ* hybridisation and treated as described above.

2.2. Larvae sorting

The larvae sampled during the live feed phase and later were sorted into stages 5–9 [40], at each specific age time point. This method divides larvae into specific developmental stages based on their myotome height. The myotome height used to define the different developmental stages is as follows: 0.65–2.0 mm (stage 5), 2.0–2.9 mm (stage 6), 2.9–4.7 mm (stage 7), 4.7–5.6 mm (stage 8), 5.6–8.2 mm (stage 9) (see also Fig. 6B). Since there is no description for staging of larvae later than stage 9, the larvae that had a myotome height of more than 8.2 mm have been described as stage >9 in this study. In general the halibut larvae in this study reached stage 5 at 59 dph, and the majority reached stage 9 at 94 dph, reaching >9 after 115 dph which corresponds to the post metamorphosis period.

2.3. Histology, immunohistochemistry (IHC) and in situ *hybridisation (ISH)*

The tissues, larvae and juveniles sampled from cohort 2 and 3, were fixed in 4% paraformaldehyde buffered with PBS at 4 °C, processed using Histokinette 2000 (Reichert–Jung) and embedded in paraffin wax within 3 days to preserve the RNA and tissue morphology. The larvae at stages 7–>9 (73 dph and later); were fixed, decalcified using Tris buffered with 10% EDTA (pH 7.2), before embedding them in paraffin wax. Sample preparation was always performed under RNase free conditions. Serial sectioning (3 μm) of larvae was performed for morphological analysis, IHC, or *in situ* hybridisation using a Leica RM 225 microtome (Leica Microsystems). Histological sections were dewaxed and stained with haematoxyline–erythrosin–safran (HES). A mouse anti-halibut IgM monoclonal antibody (Aquatic diagnostics), goat anti-mouse IgG biotin conjugate, and streptavidin-HRP (Vector laboratories) were used to detect IgM protein expression in the lymphoid organs at different developmental stages and in adult tissue samples. Normal goat serum was employed to block any non-specific interaction with mouse IgG. IgM immunostaining was performed according to the manufacturer's protocol except that we used AEC (Lab Vision Corporation) in combination with haematoxyline, instead of Vector VIP combined with methyl green during the colour development step, in order to obtain better contrast between the colour of the melanomacrophages and the substrate.

For histological studies on larvae from cohort 1, the samples were fixed in 4% paraformaldehyde, embedded in both historesin (Reichert–Jung) and paraffin wax, serial sectioned, and stained with toluidine blue or HES as described above.

Download English Version:

https://daneshyari.com/en/article/2432964

Download Persian Version:

https://daneshyari.com/article/2432964

Daneshyari.com