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Mast cells in common wolffish *Anarhichas lupus* L.: Ontogeny, distribution and association with lymphatic vessels

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ABSTRACT

The morphology, ontogeny and tissue distribution of mast cells were studied in common wolffish (*Anarhichas lupus* L.) at the larval, juvenile and adult life stages using light and electron-microscopy and immunohistochemistry. Fish were sampled at 1 day, 1, 2, 3, 4, 8 and 12 weeks post-hatching in addition to 6 and 9 months and 2 years and older. From 8 weeks post-hatching, mast cells in common wolffish mainly appeared as oval or rounded cells 8–15 μm in diameter with an eccentrically placed, ovoid nucleus and filled with cytoplasmic granules up to 1.2 μm in diameter. Granules were refractile and eosinophilic to slightly basophilic in H&E and stained bright red with Martius-scarlet-blue and purple with pinacyanol erythrosinate in formalin-fixed tissues. Mast cells stained positive for piscidin 4 and Fc ε RI by immunohistochemistry. From 1 day to 4 weeks post-hatching, immature mast cell containing only a few irregularly sized cytoplasmic granules were observed by light and electron-microscopy in loose connective tissue of cranial areas. From 1 day post-hatching, these cells stained positive for piscidin 4 and Fc ε RI by immunohistochemistry. From 12 weeks post-hatching, mast cells showed a primarily perivascular distribution and were particularly closely associated with lymphatic vessels and sinuses. Mast cells were mainly located at the peripheral border of the adventitia of arteries and veins, while they were in intimate contact with the endothelium of the lymphatic vessels. Numerous mast cells were observed in the intestine. A *stratum compactum*, as described in salmonids, was not observed in wolffish intestine, nor were mast cells confined to a separate layer, a *stratum granulosum*. Lymphatic vessels consisting of endothelium, intimal connective tissue and a poorly developed basal lamina were observed in the intestine. Scanning electron microscopy was used to compare the structure and localization of intestinal mast cells of common wolffish and rainbow trout. Scanning electron microscopy also revealed endothelial surface features and confirmed the existence of three distinctly different types of vessels in the wolffish intestine. Rainbow trout mast cell granules appeared as intact globular structures while empty vacuoles were observed in common wolffish. Mast cells were closely associated with lymphatic vessels in common wolffish, but not in rainbow trout.

1. Introduction

As described by Paul Ehrlich in 1878, mast cells are granular cells in the connective tissue of vertebrates, which stain metachromatically with aniline dyes and are localized with extremely high frequency around blood vessels in the loose connective tissues. Mast cells (MCs) have an eccentrically placed, ovoid nucleus and a cytoplasm filled with granules containing preformed, bioactive substances which are released through degranulation. In mammals, MCs are found in most organs and tissues, associated with epithelia and blood and lymphatic vessels; among other effects, their products act directly on the endothelial cells [1].

In teleosts, MCs have been described in several species [2–4]. Staining characteristics and granule contents may vary between species, but a predominantly perivascular location is constant [5]. MCs degranulate (short term response) or proliferate in numbers (long term response) in response to pathogens or irritants [6–8].

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MCs in teleosts belonging to the Order Perciformes have been shown to contain piscidins, a group of antimicrobial peptides, in addition to histamine [4,9]. In zebrafish *Danio rerio* MCs, carboxypeptidase A5 (cpa5 – an MC specific enzyme) and an IgE-like receptor (Fc ε RI) have been demonstrated [10,11]. The distribution and development of MCs in teleosts have mainly been studied in the salmonid alimentary canal, where they comprise a distinct layer, the *stratum granulosum* [12]. In Atlantic salmon *Salmo salar* L., MCs were not observed at hatching, but in small numbers surrounding developing cartilage at 1 month post-hatching, and at 8 weeks at the ventral aspect of the spinal cord and in the gill arches [13]. The time of appearance of MCs in the salmonid gastrointestinal tract seems to be closely related to the development of a dense collagenous sheath, the *stratum compactum* [14]. Salmonids and zebrafish are lower/less advanced teleosts, belonging to the Order Salmoniformes in the Superorder Protacanthopterygii and Cypriniformes in Ostariophysi respectively. Less is known about the time of appearance of MCs in more advanced teleost species lacking a *stratum granulosum*/*stratum compactum*.

Common wolffish *Anarhichas lupus* L., a higher/advanced rayfinned marine species belonging to the Order Perciformes in the Superorder Actinopterygii, is a species without a *stratum compactum*. In a study of common wolffish, fully differentiated MCs were observed in the perivascular connective tissue in stomach and intestine of juveniles and adults, but not in 1-day-old larvae [15]. In the intestine, an intimate association was observed between MCs and irregularly shaped, thin-walled vessels containing very few erythrocytes. These vessels correspond to the description of lymphatic vessels in teleosts [16,17]. There is some controversy as to whether teleost fish possess a distinct lymphatic system, or if it is a system of secondary blood vessels connected to the main cardiovascular system by arterio-arterial anastomoses [18]. In several species secondary vessels are not observed in the alimentary canal [19] and Vogel [20] states "as far as is currently known, there is no secondary veins or lymphatic-like vessels in organs of the abdominal cavity." However, recent research in zebrafish shows the existence of a real lymphatic vascular system, including intestinal lymphatics [21,22]. In the present paper, these vessels are called lymphatics.

The aim of this study was to investigate the normal development and distribution of MCs in laboratory-reared larval, juvenile and adult common wolffish through light and electron-microscopy, from a comparative point of view. The anatomy of lymphatic vessels and their association with MCs was also studied. Scanning electron-microscopy was employed to show the three-dimensional structure of vessels and cells. In addition, MCs were examined for piscidin and Fc ε RI. We also wanted to assess the use of pinacyanol erythrosinate and Martius-Scarlet-Blue (MSB) staining for identification and quantification of MCs. Pinacyanol erythrosinate visualizes mammalian MCs [23], while MSB stains MC granules in rainbow trout *Oncorhynchus mykiss* intestine a bright red (personal communication Grete Baeverfjord). Preliminary results indicated that the granule contents of common wolffish MCs were less stable than those of other species and that granule contents may be lost during fixation. The effect of different fixation protocols on granule content preservation was examined.

2. Materials and methods

The material comprised larvae, juveniles and adults of common wolffish *A. lupus*, hatched and reared under laboratory conditions at the Institute of Marine Research, Flødevigen Marine Research Station, Norway. Larvae were start-fed on a combination of zooplankton and dry pellets, while juveniles and adults were fed a commercial diet of dry pellets.

2.1. Larvae

Larvae were sampled at day 1 and 1, 2, 3, 4, 8 and 12 weeks post-hatching from a population of approximately 800 common wolffish (Table 1). The eggs were obtained from cultured wolffish broodstock, and incubated in an open egg incubation system [24]. Mean incubating temperature was 7 °C ± 0.2. Water temperature post-hatching ranged from 4.8 to 10 °C. The larvae were fed every day, using an automatic feeding system. At each sampling, 40 individuals were randomly selected among larvae displaying a normal pattern of movement and killed by decapitation.

2.1.1. Light-microscopy

For light-microscopy, the head (including pharynx and heart) and a transverse section of the trunk (incorporating the abdominal organs) were sampled and fixed for several days in 10% phosphate-buffered formalin ($n = 15$), absolute alcohol ($n = 15$) and Bouins solution ($n = 5$). Samples were dehydrated through ascending ethanol series, embedded in paraffin and sections (3–5 μm) mounted on glass slides. Formalin- and Bouins-fixed material was stained with haematoxylin and eosin (HE), and selected sections were stained with Periodic Acid Schiff (PAS), Martius-Scarlet-Blue (MSB) [25] and pinacyanol erythrosinate [23]. Absolute alcohol-fixed material was stained with toluidine blue for detection of metachromasia; in addition, selected sections were stained with HE and MSB.

2.1.2. Immunohistochemistry for piscidin and Fc ε RI

Selected sections of formalin-fixed tissue from larvae 1 day, 1, 2, 8 and 12 weeks post-hatching were deparaffinised in xylene and rehydrated.

For detection of piscidin, anti-piscidin 1 + 2 (anti-FFHH), anti-piscidin 3 (anti-HAGR) and anti-piscidin 4 were used [9,26,27]. For detection of Fc ε RI, anti-human Fc ε RIγ (Millipore, Bellerica, MA) was used [10]. Briefly, antigen retrieval was performed in 0.1 M citric acid, pH 6.0 in a microwave at 650 W for 2 × 6 min

Table 1
Weight, length and sampling of wolffish 1 day – 2½ years.

Age post-hatching N = Total number of fish	Sampled for: Light-microscopy (LM) Transmission electron-microscopy (TEM) Scanning electron-microscopy (SEM)	Mean weight in grams ± SD (range)	Mean length in mm ± SD (range)
1 day, $N = 40$	LM ($n = 35$) TEM ($n = 5$)	0.07[a]	21.8 ± 0.7 (20–23)
1 week, $N = 40$	LM ($n = 35$) TEM ($n = 5$)	0.08[a]	21.9 ± 0.6 (21–23)
2 weeks, $N = 40$	LM ($n = 35$) TEM ($n = 5$)	0.09[a]	22.6 ± 0. (20–24)
3 weeks, $N = 40$	LM ($n = 35$) TEM ($n = 5$)	0.12[a]	24.4 ± 1.5 (21–27)
4 weeks, $N = 40$	LM ($n = 35$) TEM ($n = 5$)	0.15[a]	25.5 ± 1.3 (23–28)
8 weeks, $N = 40$	LM ($n = 35$) TEM ($n = 5$)	0.31[a]	33.2 ± 2.0 (28–37)
12 weeks, $N = 40$	LM ($n = 35$) TEM ($n = 5$)	0.79[a]	46 ± 4.5 (34–56)
6 months, $N = 20$	LM ($n = 15$) TEM ($n = 5$)	10.8 ± 2.7 (5.8–14.8)	100 ± 10 (85–115)
9 months, $N = 5$	TEM ($n = 5$)	18.6 ± 2.2 (15.73–21.06)	133 ± 6 (125–140)
2 years, $N = 3$	TEM ($n = 3$) SEM ($n = 3$)	336.3 ± 31.5 (300–355)	328 ± 8 (320–335)
2½ years $N = 5$	TEM ($n = 5$) SEM ($n = 5$)	303 ± 54.8 (232–378)	317 ± 21 (285–350)

[a] Due to the low weight of the larvae, they were weighed as a group and mean weight calculated.