

lower vertebrates, there is, as yet, only scarce information available regarding this inter-system communication. Recently, we have made a start through studies on the role of the pituitary adenylate cyclase-activating peptide (PACAP) in innate and adaptive immunity in fish. We have shown that administration of this neuropeptide increases humoral immune parameters, such as, lysozyme, nitric oxide synthase-derived metabolites and immunoglobulin M in fish larvae and juveniles. This immunological status was correlated with higher growth hormone concentration in serum and with an improvement of the fish antioxidant defence mechanisms. Current work provides new insights about the effects of PACAP on the fish immune system. It demonstrated for the first time both the occurrence of the two PACAP transcriptional variants (PACAP and PRP/PACAP) together with their receptors (PAC-1, VPAC-1 and VPAC-2) in diverse lymphoid organs of the salmonid fish *Oncorhynchus mykiss* and *Salmo trutta*. Additionally, their expression levels were assessed in spleen, kidney, head kidney and spleen leukocytes, and in the monocyte/macrophage cell line RTS11 at different time points after infection with important pathogens for aquaculture: such as the bacteria *Yersinia ruckeri* and the viruses viral hemorrhagic septicemia virus (VHSV) and infectious pancreatic necrosis virus (IPNV). The results disclosed a differential regulation of the PACAP transcripts and their receptors after infections. These findings added PACAP and its receptors to the growing list of mediators shared by the nervous, endocrine and immune system in fish, and suggest a possible role of these molecules in antiviral/antibacterial immunity. To support the previous hypothesis, a direct action of PACAP on the regulation of different immune genes and cytokines in fish lymphoid tissue was evaluated. We have observed that PACAP increases the IL-1 β , TNF α , IL-15, Mx, INF gamma and TLR9 mRNA levels in fish peripheral blood leukocytes *in vitro*. This effect was associated with its ability to enhance the MHC-II, CD4 co-receptor and IgM transcripts. All this information together would significantly add to the understanding of fish physiology needed to obtain enhanced health of fish in aquaculture.

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First comparative study on the presence of antimicrobial peptides in infected and uninfected intestine of *Perca fluviatilis* (PISCES)

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Abstract

The ability to avoid infection depends on animal mechanisms of innate immunity. The innate immune system is responsible for the primary response and one type of granular cells, mast cells (MCs) has been reported to play a critical role as part of the defence function against pathogens and evidence for its involvement in the immune system of fish is growing. Antimicrobial peptides (AMPs) are widely distributed in nature and are considered to be ancestral components in the evolution on innate immunity. Several types of antimicrobial peptides (AMPs) have been isolated from fish and they are present in mucosal surfaces and in immune cells such as MCs.

One type of AMPs expressed by fish MCs is piscidins. Twenty-four perch, *Perca fluviatilis* (22,95 \pm 7,48, mean total length \pm S.D.) from Lake Piediluco (Centre Italy) were caught by professional fishermen using a gill net. Fourteen pike were infected with an intestinal parasite, *Acanthocephalus lucii*. The pathological changes and the immune response induced by worms were assessed using histological, immunohistochemical and ultrastructural techniques. The parasites penetrate not deeply into the intestinal wall and induce slight inflammation to the organ. The main

damage caused by worms to the host digestive tract was the destruction of the mucosal epithelium covering the villi next to the parasites site of attachment, which included necrosis and degeneration. Immunostaining of the perch intestine with antibodies against the peptide antibiotics piscidin 3 (anti-HAGR) and piscidin 4 (anti-5.3-02-3A) showed sub-populations of MCs that were positive. Piscidin positive MCs were observed especially among the epithelial cells of the intestine but also within the submucosal layer. In both uninfected and infected perch, the number of MCs positive to piscidin 4 was higher than those immunoreactive with piscidin 3 (ANOVA, $p < 0.05$). For both piscidins, there was no significant difference in the number of positive MCs between infected and uninfected intestine (ANOVA, $p > 0.05$). The immunostaining intensity was also compared in infected and healthy fish but no distinct differences were noticed (ANOVA, $p > 0.05$). Our study provides the first evidence of the presence of piscidins in digestive tract of a fish infected with intestinal helminths.

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Gadus morhua L: Acute phase responses in larvae and juveniles

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Abstract

Atlantic cod depends solely on innate immunity for the first 10–12 weeks after hatch. This is a stressful time with metamorphosis, fast growth and start of feeding. Studies of the developing immune system are important for understanding its role in early development and assessing various treatments. Many studies have shown the importance of innate immune responses for mature cod, observations that were recently underlined by the fact that the MHC Class II genes are lacking in this species.

Cod larvae and juveniles were sampled: 1) larvae 2 – 85 days post hatch (d.p.h.), placed in formaldehyde, embedded in paraffin and sections prepared for immuno-histochemistry analysis using specific antibodies for cod pentraxins CRP-PI and CRP-P II, 2) larvae 0 – 27 d.p.h., were sampled into RNAlater for real time quantitative PCR (RT-qPCR) analysis of CRP-PI, CRP-P II and transferrin, 3) cod juveniles (85 g) were injected i.m. with turpentine oil. Head kidney and spleen were sampled and placed in RNAlater after one, 24, 72 and 168 hours, for RT-qPCR analysis of CRP-PI, CRP-P II, C3, ApoLP AI, transferrin, IL-1 β , cathelicidin and hepcidin.

The presence of CRP-PI and CRP-P II was demonstrated in liver at first sampling using immunohistochemistry. Fifty d.p.h., CRP-PI had almost disappeared while CRP-P II was still present. RT-qPCR analysis showed that the expression of CRP-PI diminished in the first week after hatch, but increased in weeks three and four. Expression of CRP-P II cumulated two d.p.h, decreased in the following week and increased in weeks three and four. The gene expression of transferrin rose steadily after hatch, reaching peak expression on day 15. There was a transient lowering on day 17 for all parameters, coinciding with the time when *Artemia* was added to the feed for the first time. In the kidney of juvenile fish, CRP-PI, CRP-P II and ApoLP AI expression was increased one hour after treatment. After 24 hours, only hepcidin expression in the spleen was increased. By 72 h, transferrin, IL-1 β and cathelicidin expression was raised in both organs. Transferrin only showed increased expression in kidney after 168 h. Transferrin, rather than pentraxins, might be a potential health marker in larvae as well as juveniles.

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