

salmon. Thus, salmon *il-4/13A* gene was synthesized and cloned in pET15b and recombinant IL-4/13A was produced in *E. coli*. rIL-4/13A was purified, and the activity verified *in vitro*. *In vivo* analysis of the IL-4/13A biological activity was performed in salmon receiving the recombinant cytokine. Effects were compared with those of a control group receiving saline. Transcription expression of marker genes for Th1 and Th2 responses was analyzed in the spleen and head kidney of treated and control fish. Results showed that IL-4/13A induced the expression of its own gene, GATA-3, IFN- γ and MHC class II in the head kidney of fish. No changes were observed for IL-10 in the head kidney. Expression did not change for any of the genes tested in the spleen of the IL-4/13A-treated fish. In regard to the receptors, *γ C1*, *il-4 α* , *il-13 α 1a*, *il-13 α 1b* and *il-13 α 2a* transcripts were detected in most lymphoid and non-lymphoid tissues. Full CDS sequences were cloned from RNA of head kidney leukocytes and then sequenced. Structural analysis of the predicted receptor proteins and 3D models allowed the identification of domains and motifs that are conserved in most IL-4 and 13 receptor chains. Interestingly, IL-4/13A upregulated the transcriptional expression of the receptors in the spleen but not in the head kidney of salmon. Results showed that the IL-4/13 system, which in superior vertebrates induces the Th2 responses, is also conserved in Atlantic salmon and seems to control the expression of key genes involved in adaptive immune responses.

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O-082.

Iron overload alters the immune response in Atlantic salmon and increases the susceptibility to *Piscirickettsia salmonis* infection

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Abstract

Iron is a vital element for life, but high levels can produce deleterious effects for the organism's development. In mammals it has been demonstrated that iron has an important role in immune system. However, iron overload can increase the production of free radicals, inducing negative effects in immune cells. The excess of iron accumulation has also been associated as a key factor for bacterial pathogenesis. Despite the importance of iron regulation in the immune system, the effects of iron overloads in fish have poorly been studied. The aim of this study was to evaluate the transcriptional changes of Atlantic salmon exposed to iron overload and challenged to the intracellular bacterium *Piscirickettsia salmonis*. Here, fish were injected with 1 and 5 mg of iron dextran and after eight days injected with *P. salmonis*. Samples of head kidney, liver and spleen were collected for transcriptome analysis at 0 and 8 days post-injection and 12 days post-bacterial challenge. GO enrichment analysis showed a high number of transcripts differently expressed with association to iron transport, response to oxidative stress and immune response. Notably, fish exposed to iron overload showed downregulation of immune-related genes. Furthermore, histological analysis conducted in infected fish groups showed clinical alterations in salmon previously overloaded with iron. GO enrichment analysis in infected fish showed high abundance of genes associated with immune process regulation, negative regulation of cytokines and regulation of apoptotic process. These biological processes were mainly modulated in fish exposed to iron. This study evidences the effects of iron overload associated to fish immune response, revealing novel insights about the importance of iron regulation and its impact over the immune response in teleost fish.

Keywords: Iron overload, Atlantic salmon, transcriptome analysis, immune modulation, *P. salmonis*

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O-083.

The expression of TRPV channels, prostaglandin E2 and pro-inflammatory cytokines during behavioural fever in fish

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Abstract

A fever, or increased body temperature, is a symptom of inflammation, which is a complex defense reaction of the organism to pathogenic infections. After pathogens enter the body, immune cells secrete a number of agents, the functions of which stimulate the body to develop a functional immune and fever response. In mammals it is known that PGE2 is the principal mediator of fever. The extent to which PGE2 and other pro-inflammatory cytokines such as TNF- α , IL-6, or IL-1 β could be involved in the induction of behavioral fever in fish remains to be clarified. Several members of the transient receptor potential (TRP) family of ion channels have been implicated as transducers of thermal stimuli, including TRPV1 and TRPV2, which are activated by heat. Here we show that members of the TRP family, TRPV1 and TRPV4, may participate in the coordination of temperature sensing during the behavioral fever. To examine the behavioral fever mechanism in *Salmo salar* an infection with IPNV, infectious pancreatic necrosis virus, was carried out by an immersion challenge with 10 x 10⁵ PFU/mL of IPNV. Behavioral fever impacted upon the expression levels of both TRPV1 and TRPV4 mRNAs after the viral challenge and revealed a juxtaposed regulation of TRPV channels. Our results suggest that an increase in the mRNA abundance of TRPV1 is tightly correlated with a significant elevation in the expression of proinflammatory cytokines (IL-1 β , IL-6, TNF- α and PGE2) in the Pre-Optic Area (POA) and cytokine release in plasma. Together, these data indicate that the reduction of TRPV4 expression during behavioral fever may contribute to the onset of behavioral fever influencing movement toward higher water temperatures. Our data also suggest an effect of TRPV channels in the regulation of behavioral fever through activation of EP3 receptors in the central nervous system by PGE2 induced by plasma-borne cytokines. These results highlight for first time in mobile ectotherms the key role of pro-inflammatory cytokines and TRPV channels in behavioral fever that likely involves a complex integration of prostaglandin induction, cytokine recognition and temperature sensing.

Keywords: Ectotherm, Behavioral fever, Cytokine, TRP channels and Virus

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O-084.

Characterization of CD37+ T lymphocytes in the teleost *Dicentrarchus labrax* L.

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Abstract