

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 CD3?+ T lymphocytes in the teleost *Dicentrarchus labrax* L.

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Abstract

CD3 chains play key roles in the development and activation of T cells in higher vertebrates. In the present study, a complete cDNA sequence of CD3 ϵ chain was identified from a sea bass gills transcriptome. Realtime PCR was employed to investigate the basal quantitative levels of CD3 ϵ in tissues and lymphoid organs of sea bass juveniles, with the highest expression found in the thymus followed by gut, spleen, head kidney (HK), peripheral blood leukocytes (PBL) and gills. In vitro stimulation of HK leukocytes with either T-cell mitogen PHA or sea bass recombinant IL-2, resulted in a significant increase of CD3 ϵ transcripts compared to control cultures. The CD3 ϵ cytoplasmic tail region was also identified and used to select three peptides as immunogens in rabbits, in order to produce a polyclonal antiserum. The antibody, named Ra CD3 ϵ 1, recognized the immunization peptides in ELISA and stained a band of the expected size in WB at ca. 17 kDa. The distribution and number of CD3 ϵ + lymphocyte population in the lymphoid organs, mucosal tissues and PBL were addressed in healthy fish by IHC, IIF and flow cytometry, with relatively high percentages of these cells detected among thymocytes, HK, gill and gut leukocytes and PBL, while moderate percentage were found in splenocytes. At the microscope the IIF-positive cells had the typical lymphocyte morphology and a subset of uniquely stained CD3 ϵ + IgM- cells fit the expected T cell profile. Oral stimulation with a *Vibrio anguillarum* vaccine increased the CD3 ϵ expression level in HK leukocytes, suggesting that T CD3 ϵ + lymphocytes may play important roles in the systemic protection against pathogens. Finally, the in vivo modulation of CD3 ϵ + T intestinal lymphocytes was investigated in fish fed on diets where 50% or 100% fish meal was replaced with the marine-water microalgae *Nannochloropsis* sp. biomass. IHC revealed a significant enhanced density of T CD3 ϵ + cells in the mucosa of mid intestine compared to fish fed on a control diet. These data suggest that CD3 ϵ + T lymphocytes may be involved in dietary intestinal immune responses.

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Keywords: CD3 ϵ sea bass, polyclonal antibody, immune responses, T cells

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O-085.

Identification and characterization of plasma-like cells in grass carp

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Abstract

In mammals, plasma cells are well characterized, and CD40L and IL-21 can induce plasma cell differentiation and immunoglobulin production. In this study, we developed monoclonal antibody against grass carp IgM and identified two different IgM+ B cell subsets, namely small IgM+ B cells and large IgM+ B cells. The large IgM+ B cells were further identified as plasma-like cells because they showed gene expression patterns similar with those of human plasma cells and a great capacity to secrete IgM. The small and large IgM+ B cells from either healthy grass carp or the fish stimulated with LPS or Poly (I:C) showed similar phagocytic activity. Recombinant CD40L or IL-21 alone could induce plasma-like cell generation and IgM secretion. Compared with CD40L or IL-21 alone, the combination of CD40L and IL-21 had greater effect on IgM secretion, but not on plasma-like cell generation. These results suggest that plasma-like cells in teleost fish have their own features, such as expressing cell surface IgM and possessing phagocytic activity.

Keywords: B cell, plasma-like cell, CD40L, IL-21, grass carp

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O-086.

Formulation of new adjuvants to be used in fish vaccines

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Abstract

Vaccination is considered the best way of controlling infectious diseases in aquaculture. However, most of the adjuvants used in aquaculture, especially oil-based adjuvants, cause damage to fish, with consequent retardation of growth and negative effects on the welfare of the fish. In this study we developed and tested several adjuvants formulated with carboxymethylcellulose, ulvan or chitosan as the main components. We prepared and tested five formulations, including carboxymethylcellulose-methacrylate gel, ulvan-methacrylate gel, ulvan gel, ulvan-chitosan gel and chitosan gel. All formulations were tested in turbot (*Scophthalmus maximus*) and were administered alone or in combination with particulate antigen obtained from the ciliate parasite *Philasterides dicentrarchi*. Control groups of fish were injected with PBS, antigen or a vaccine containing an oil-based adjuvant (positive group). Fish were injected i.p. on days 0 and 30 with the appropriate vaccine formulation, and on day 60 the fish were examined for intraperitoneal lesions and to determine growth and specific serum immunoglobulin levels (IgM). None of the formulations caused important internal lesions (only small adhesions between internal organs and the peritoneal wall at the injection site), and they did not affect fish growth. Among all the formulations, the chitosan gel yielded the best response in terms of fish serum antibody levels. We also analyzed the immune response generated by the formulations in CD1® IGS mice. Mice were injected i.p. with FCA, aluminum hydroxide, carboxymethylcellulose-methacrylate gel, ulvan-methacrylate gel, ulvan gel, ulvan-chitosan gel or chitosan gel and *P. dicentrarchi* antigen. The best responses in terms of serum antibody levels were obtained with ulvan-methacrylate gel and chitosan gel. The values obtained were similar to those obtained in response to FCA and significantly lower than those obtained in response aluminum hydroxide. In addition, we compared the polarization of th1/th2 response in mice injected with these adjuvants. The results obtained suggest that alternative adjuvants, which induce a good immune response and do not cause important internal lesions, can be formulated for inclusion in fish vaccines.

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Keywords: Turbot, vaccine, *Philasterides dicentrarchi*, ulvan, chitosan, immune response

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O-087.

Formulation of *A. salmonicida* adjuvanted vaccine for rainbow trout : Impact of the adjuvant oil origin

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