O-079.

Morphological properties of gill-epithelial antigen sampling (GAS) cells in rainbow trout

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Abstract
Bath-vaccination is a cost-effective technique to apply vaccines to fish. However, mechanisms of antigen uptake and immune recognition on the mucosal surfaces of fish are largely unknown. We have previously reported that bacterial vaccine antigens are taken up by gill epithelial antigen sampling (GAS) cells. GAS cells are characterized by their binding capacity for the lectin *Ulex europaeus* agglutinin 1 (UEA-1), and their properties of taking up inactivated *Aeromonas salmonicida* subsp. *salmonicida* (*A.s.s.*) bacteria. In addition, GAS cells express genes and molecules that are typical for M cells but also for antigen processing and presentation. Recently, we have developed a monoclonal antibody (mAb) against GAS cells (2B4-1) that specifically binds to UEA- 1+ *A.s.s.*+ cells. In the present study, we aimed to investigate the morphological properties of GAS cells using mAb 2B4-1 by light and transmission electron microscopy (TEM).
The epithelial cells of the gills of rainbow trout were dispersed using 10 mM EDTA and stained with UEA-1 and mAb 2B4-1. UEA-1+ 2B4-1+ cells were sorted by flow cytometry and subjected to May-Grünwald Giemsa staining. The sorted cells were also embedded in epoxy resin and analyzed by TEM. Further, the gills removed from a rainbow trout were embedded in Lowicryl K4M and the ultrathin sections were subjected to immune electron microscopy using mAb 2B4-1.
May-Grünwald Giemsa staining revealed that there are two cell types in UEA-1+ 2B4-1+ cells: one with a fragmented or condensed nucleus and many vacuoles, and a second with a relatively large nucleus. In TEM, the first phenotype of the UEA-1+ 2B4- 1+ cells showed spines on their surface, an electron dense cytoplasm, and numerous lysosome- or phagosome-like vacuoles. The second phenotype showed a rather low- density cytoplasm and some vacuoles in the cytoplasm, suggesting that this cell type is not active in terms of antigen uptake and processing. Immune electron microscopy revealed that mAb 2B4-1+ cells located on the surface of the gill epithelial layer. The mAb signals were found on the spines and cytoplasm of UEA-1+ 2B4-1+ cells.
Taken together, these morphological properties of GAS cells in rainbow trout support our previous observations that teleost GAS cells have functions in antigen processing

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O-080.

GCRV triggers but major outer capsid protein VP4 inhibits RIG-I mediated interferon response

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Abstract
Grass carp (*Ctenopharyngodon idella*) is a very economically important aquaculture species, however, grass carp reovirus (GCRV) has caused severe epidemic outbreaks of hemorrhagic disease and tremendous mortality in grass carp industry. RIG-I-like receptors (RLRs) are critical cytosolic sensors in antiviral immunity, coupling detection of virus infection to interferon (IFN) production. In the present study, mRNA expressions of RLRs, including retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2), were significantly up-regulated after GCRV infection. Extensive type I IFN response was activated by RIG-I- and MDA5-induced IFN regulatory factor (IRF) 3 (IRF3) and IRF7 mRNA expressions and total phosphorylation levels. Meanwhile, LGP2 worked at upstream of RIG-I and MDA5, restraining K63- and K48-linked ubiquitination of RIG-I and MDA5 in various degrees. It inhibited synthesis and phosphorylation of IRF3/7, leading to reduce mRNA levels and promoter activities of IFNs and NF-κB_s_._ _G_C_R_V_ _major outer capsid protein VP4 was found to localize in lysosome, early endosome and endoplasmic reticulum. To investigate the proteins that interact with VP4, intact VP4 protein was employed and immunoprecipitation (IP) was performed using VP4 polyclonal antibody. According to the subsequent LC MS/MS, RIG-I was obtained and verified to interact with VP4 by co-IP and bimolecular fluorescence complementation (BiFC). VP4 overexpression observably declined mRNA expressions and promoter activities of RIG-I and downstream key genes in RLR pathway, including IFNs. As a consequence, antiviral effectors were significantly suppressed in mRNA levels and GCRV replication notably increased, resulting in conspicuously intensified cytopathic effect. Knockdown of VP4 obtains opposite effects. Furthermore, transcriptome sequencing of VP4 overexpression CIK cells was carried out, and the results indicated that VP4 may trigger MyD88-dependent toll-like receptor (TLR) signaling pathway. These results collectively revealed that GCRV infection activates RLR pathway, however, VP4 associated with RIG-I suppresses downstream IFN response to evade antiviral immunity. This study lays the foundation for the further anti-dsRNA virus mechanism research of RIG-I in teleost and the strategy of GCRV for evading the host IFN response.
Keywords: Grass carp reovirus (GCRV); Major outer capsid protein; RIG-I-like receptors (RLRs); Interferon (IFN); Grass carp (*Ctenopharyngodon idella*)

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O-081.

IL-4/13A and its receptor system in Atlantic salmon (*Salmo salar*): Upregulation of key genes involved in adaptive immunity

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Abstract
Interleukin (IL)-4 and IL-13 play a central role for T helper 2 immune response in mammals. Cell signaling is mediated by the type I receptor formed by IL-4Rα and γC chains, and the type II receptors formed by IL-4Rα and IL-13RF0611. In fish, IL-4 and IL-13 related genes have been found in several fish species, including rainbow trout and Atlantic salmon. In these salmonid species, three paralogues of the IL-4 and IL-13 cytokines have been reported, *il-4/13a*, *il-4/13b1* and *il-4/13b2*. In regard to the receptors, two paralogues of each IL-4/13 receptor chains have been identified in rainbow trout. In Atlantic salmon, we and others have identified 5 genes named *γc1, il-4rα, il-13rα1a, il-13rα1b*, and *il-13rα2*. Since Atlantic salmon is an important aquacultured fish species, and also a good model for the study of evolution of the immune system, the aim of this work was to get new insights into the functional role of IL-4/13A and their receptors in

salmon. Thus, salmon *il-4/13A* gene was synthetized 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, *γc1, il-4rα, il-13rα1a, il-13rα1b and il-13rα2a* 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 overdoses in fish have poorly been study. 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 salmons 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-1β 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-1β, 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