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