be a new target for the strategy of disease control and vaccine development.

Keywords: Thrombocytes, Interferon, TLRs, innate immunity, interferon regulatory factor

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O-105.

Activation of DExD/H-box RNA helicases during infection of zebrafish and common carp with spring viraemia of carp virus (SVCV) and chum salmon reovirus (CSV)

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Abstract
The innate immune system detects viral infection predominantly by sensing viral nucleic acids in infected cells. The main sensors of viral RNA in cytoplasm are members of RIG-I-like receptors (RLR) such as RIG-I, MDA5 and LGP2. Recently, several non-RLR DExD/H-box RNA helicases have been also shown to play important role in sensing of viral nucleic acids in the cytoplasm and to activate downstream signaling pathways leading to type I interferons (IFN) production and anti-viral response in mammals. However, the mechanisms of action of these RNA helicases are still not fully understood, and their role in the anti-viral immune response in fish has not been studied. In the present work we aimed to study, for the first time in fish, the anti-viral role of DExD/H-box RNA helicases: DDX1, DDX3, DHX9, DDX21 and DHX36 during viral infection of two cyprinid fish: zebrafish (*Danio rerio*) and common carp (*Cyprinus carpio*). We studied expression of DExD/H-box RNA helicases, type I IFNs and antiviral proteins in zebrafish during infection with spring viraemia of carp virus (SVCV) and chum salmon reovirus (CSV) both *in vitro* (ZF4 cell line) and *in vivo*. Moreover, expression of studied genes was analyzed in common carp during *in vivo* infection with SVCV. *In vitro* studies of both viral models demonstrated a significant up-regulation of the expression of IFN type I genes in ZF4 cell line. However, SVCV did not induce changes in the gene expression of DExD/H-box RNA helicases, up-regulation of the expression of *ddx3*, *dhx9* and *ddx21* was observed in ZF4 cells upon CSV infection. *In vivo* SVCV infection of zebrafish induced a significant up-regulation of *ddx1* and *dhx36* expression while CSV infection induced a significant up-regulation of *ddx1* and *dhx9* expression. In both infection models an up-regulation of the expression of IFN type I genes and interferon stimulated genes (ISG) *mxa* and *vig-1* was observed. In common carp SVCV infection resulted in up-regulation of the expression of *ddx1*, *dhx9* and *ddx21*, IFN type I and *vig-1*. In both zebrafish and common carp, the up-regulation of the gene expression of DExD/H-box RNA helicases correlated with the increase of the viral load and in most of the cases preceded up-regulation of the IFN type I genes expression. In conclusions our data suggest that non-RLR DExD/H-box RNA helicases might be involved in fish in sensing of viral infection and induction of anti-viaral immune response.
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Keywords: DExD/H-box RNA helicases, interferons, *vig-1*, SVCV, CSV

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O-106.

The fish coagulation system could help to prevent infection by the ciliate parasite *Philasterides dicentrarchi*

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Abstract
In addition to its role in hemostasis, the coagulation system is involved in defence against pathogens in invertebrates and vertebrates. In mammals, the coagulation system has been shown to participate in entrapping pathogens and activating the early immune response. Although many studies have described different components of the fish coagulation system, there is a lack of information about the importance of the system in host defence against pathogens. In the present study, we showed that injecting turbot (*Scophthalamus maximus*) with the pathogenic ciliate *Philasterides dicentrarchi* generates the formation of macroscopic intraperitoneal clots in the fish. The clots contained abundant, immobilized ciliates, many of which were lysed. We observed that the plasma clots immobilize and kill the ciliates in vitro. However, fish plasma treated with a tetrapeptide known to inhibit fibrinogen/thrombin clotting in mammals killed *P. dicentrarchi* slightly faster than the untreated plasma, although the overall mortality rate was similar. We also found that kaolin, a particulate activator of the intrinsic pathway in mammals, accelerates plasma clotting in turbot. PMA-stimulated neutrophils, living ciliates and several ciliate components (such as cilia, proteases and DNA) also displayed procoagulant activity in vitro. In addition to generating clots in the peritoneal cavity, i.p. injection of ciliates generated massive migration of neutrophils to the peritoneal cavity, with the formation of large cell aggregates and of numerous fibrin-like fibres in the peritoneal exudate, many of which were associated with peritoneal leukocytes and ciliates. Expression of the CD18/CD11b gene, an integrin associated with cell adhesion and the induction of fibrin formation, was upregulated in the peritoneal leukocytes. In conclusion, the results of the present study suggest that the fish coagulation system plays an important role in immobilizing *P. dicentrarchi* during early moments of infection and appears to be an important component of the protection against this pathogen in fish.
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Keywords: Coagulation system; Complement; Fish; Neutrophils; *Philasterides dicentrarchi*; Plasma

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O-107.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is lethal to *Flavobacterium psychrophilum* through membrane permeabilization and by priming immune function in rainbow trout macrophages

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Abstract
Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional neuropeptide that is widely distributed in mammals and is capable of performing roles as a neurotransmitter, neuromodulator and vasodilator. This polypeptide belongs to the glucagon/secretin superfamily, of which some members have been shown to act as antimicrobial peptides in both mammalian and aquatic organisms. In teleosts, PACAP has been demonstrated to have direct antimicrobial activity against several aquatic pathogens, yet this phenomenon has never been studied throughout a live bacterial infection. The present study focuses on the influence of PACAP on the rainbow trout monocyte/macrophage-like cell line, RTS11, when exposed to the coldwater bacterial pathogen *Flavobacterium psychrophilum*. PACAP was shown to have direct antimicrobial activity on *F. psychrophilum* when grown in both cytophaga broth and cell culture media (L-15). Further, the ability of teleostean PACAP to permeabilize the membrane of an aquatic pathogen, *F. psychrophilum*, was revealed for the first time. The viability of RTS11 when exposed to PACAP was also observed using a trypan blue exclusion assay to determine optimal experimental doses of the antimicrobial peptide. Interestingly, when RTS11 was pre-treated with PACAP for 24 hours before experiencing exposure to live *F. psychrophilum*, growth of the pathogen was severely inhibited in a dose-dependent manner when compared to control cells receiving no PACAP pre-treatment. Relative expression of pro-inflammatory cytokines and PACAP receptors was also observed in RTS11 following PACAP exposure alone and in conjunction with live *F. psychrophilum* challenge. These qRT-PCR findings revealed that PACAP may have a synergistic effect on RTS11 immune function. The results of this study provide evidence that PACAP has immunostimulatory activity on rainbow trout immune cells as well as direct antimicrobial activity against aquatic bacterial pathogens such as *F. psychrophilum*. As there are numerous pathogens that impact the aquaculture industry, PACAP may stimulate the teleost immune system while also providing an efficacious alternative to antibiotic use.
Keywords: Rainbow trout, antimicrobial peptide, PACAP, RTS11, *Flavobacterium psychrophilum*

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O-108.

Efficient and long-lasting protection against the pacific oyster mortality syndrome through antiviral immune priming

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Abstract
The major economic and environmental consequences of recurring mortalities affecting the Pacific oyster *Crassostrea gigas* have initiated many research projects aiming at understanding these phenomena. The solutions anticipated to deal with these mortalities are mainly based on mass selection breeding programs but preventive treatments are still lacking. However, over the last decade, studies have been accumulating revealing the adaptive capabilities of innate immunity, the only component of defense mechanisms in invertebrates. Numerous findings have shown that a wide range of invertebrates can develop innate immune memory (also called immune priming) leading to improved survival during a second encounter with a pathogen. In this context, we undertook to study the possibilities of acting against mortalities by stimulating immune capacities of oysters.
In the present study, we show that the exposure of oyster juveniles to an immunostimulant (a viral mimic called poly (I: C)) can lead to enhanced survival capacities (up to 100%) following OsHV-1 infection or during a mortality episode in the field. That protection is specific to viral protection as poly(I:C) fails to protect oyster against a pathogenic bacteria. We also show that this priming phenomenon is durable as it can last more than 4 months suggesting for the first time the existence of mechanisms of immune memory in this invertebrate species. Finally, analysis of the molecular pathways underlying that protection using dual RNAseq, revealed that priming was based on the triggering of a strong and sustained antiviral response limiting replication of the virus, thus allowing the protection of oysters on the long term. Altogether these results bring new insights into the oyster capacities to build an innate immune memory, its adaptive capacities and provide a platform to further explore novel strategies to help mitigate disease threats upon marine bivalves.
Keywords: Immunity, antiviral, OsHV-1, priming, oyster

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O-109.

The immune recognition mechanisms in mollucs

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Abstract
Immune recognition is the first step of immune response, which plays key role in immune protection in all organisms. Since the proposal of pattern recognition theory, numerous pattern recognition receptors (PRRs) have been reported, especially in molluscs where expansive PRRs have been identified by genomic prediction. Taken the model bivalve oyster as example, oysters are constantly threatened by the invasion of pathogens and they have developed a sophisticated repertoire of PRRs to recognize diversiform microorganisms. So far, seven PPRs families, such as peptidoglycan recognition proteins (PGRPs), lectins (including C-type lectins, galectins and sialic acid-binding Ig-like lectins), toll-like receptors (TLRs), C1q domain containing proteins (C1qDCs), Gram-negative binding proteins (GNBPs), scavenger receptors (SRs) and fibrinogen-related proteins (FREPs) have been identified in oysters. What's more, some novel PRRs, such as DM9 domain containing proteins (DM9CPs), Caspases, interleukin 17 (IL-17) and arginine kinase have also been characterized from oysters, which expands our knowledge of PRRs in invertebrates. These various PRRs have been partially validated to be different in recognition specificity, down-stream signal pathway and immune effects. Most of the PRRs serve as multi-functional proteins, not only in immune recognition, but also in the elimination of invading microbes. In addition, these PRRs differentially expressed in mucosal immune tissues and systemic circulatory system where immune recognition taking place. And different effects could be induced by mucosal and systemic immune recognition, such as immune memory or immune tolerance. These results uncovered in molluscs have expanded our knowledge about the classical pattern recognition theory, and also provided theoretical basis for vaccine development in mollusc breeding-species in the future.