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Viral hemorrhagic septicemia virus (VHSV) is a major concern in aquaculture, as it can cause significant mortality in juvenile fish. Whilst no cure for VHSV currently exists, several vaccination approaches are being developed to protect against the virus. In this study, we investigated the potential of liponanoparticles (LNPs) to serve as a delivery system for mRNA vaccines combined with immunomodulators targeting TLR2, 3 and 7/8 in rainbow trout. Specifically, Pam3 and 3-MO52 agonists of these TLRs, were loaded into micelles and co-injected alongside LNP vaccines. This study included a challenge against VHSV, and found that the LNP mRNA vaccine alone was able to provide significant protection, comparable to that of attenuated VHSV, which has previously been shown to be 100% effective. We performed a comparative trial using DNA vaccines co-injected with micelles containing 3-MO52. We characterised specific immune responses to the vaccines and micelles in various tissues, examining both systemic and mucosal responses, and compared them to the DNA vaccines previously developed as a benchmark. We also evaluated the correlates of protection using serum neutralization assays, to determine if host antibodies are specifically produced to offer protection against VHSV and whether the protection offered is antibody-dependent. These results suggest that the LNP mRNA vaccine approach could be an efficient strategy for preventing VHSV infections in salmonids.

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ANTIVIRAL MECHANISM OF INTERFERON-LIKE AND RNAI SYSTEM IN OYSTER

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The interferon (IFN) system serves as the first line of immune defence against viral infection, which is generally recognized to originate from earliest jawed vertebrates. While recent studies have identified the IFN-like antiviral cytokines in invertebrates, such as Vagos from arthropod and IFN-like protein (*Cg*IFNLP) from oyster *Crassostrea gigas*. *Cg*IFNLP strongly responded to Poly (I:C) stimulation, and its expression is dependent on the *Cg*cGAS/STING-TBK1-IRF regulatory axis. Three interferon regulatory factor (IRF) members and one IRF binding protein (IRF2BP) were identified in the oyster, all of them involved in regulation of *Cg*IFNLP expression. The *Cg*IFNLP was able to interact with its receptors (*Cg*IFNR3 and *Cg*IFNLPR-1) to induce the expression of interferon-stimulated genes (ISG), such as *Cg*Mx1, *Cg*IFI44L and *Cg*Viperin. Therefore, the *Cg*IFNLP showed relatively conserved antiviral immune response in oyster. The RNAi is considered as main antiviral mechanism in invertebrates, which is also identified in oyster and showed antiviral function. Recently we found the possible crosstalk of RNAi and IFN-like system in oyster. The r*Cg*IFNLP stimulation could induce the expresion of RNAi components, including Dicers and Agos. And the expression level of *Cg*IFNLP was significantly decreased in the Dicer/Ago-RNAi oysters. Interestingly, the PIWI homologue in oyster showed negative regulation on *Cg*IFNLP expression. These results suggest the existence of IFN-like and RNAi system in oyster, and hinting their possible crosstalk underly the antiviral immune response in mollusca.

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REGULATORS OF NF-κB ACTIVATION: CHARACTERISATION OF THE LARGE NF-κB INHIBITORS FAMILY IN SALMONID FISH

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NF-κB inhibitors (IκB) control signalling of the family of NF-κB which plays a major role in immune and stress responses. In the NCBI database, six *nfkbia*, two *nfkbie*, two *nfkbid*, two *nfkbiz* and two *bcl3* genes in rainbow trout were identified. Salmonid fish contain three paralogous nfkbia genes, two of which share a high degree of sequence similarity, whereas the third gene is more divergent. Additionally, the product iκbα of this specific *nfkbia* gene groups with the human IκBβ in a phylogenetic analysis and is significantly less expressed than the structurally more closely related *nfkbia* paralogs. Overall, iκbβ has most likely not been lost in salmonid genomes, but has been mislabelled as iκbα. Two gene isoforms coding for iκbα (*nfkbia*) and iκbε (*nfkbie*) had high transcript concentrations in the immune tissues and in a cell fraction enriched with granulocytes, monocytes/macrophages and dendritic cells from the head kidney of rainbow trout. After zymosan stimulation of salmonid CHSE-214 cells, the expression of *nfkbia* together with inflammatory markers *il1b* and *il8* were significantly upregulated. Overexpression of iκbα and iκbε in CHSE-214 cells demonstrated a dose-dependent inhibition of the basal and stimulated activity of a NF-κB promoter, indicating their participation in immune regulatory processes. This study presents the whole salmonid family of NF-κB inhibitors and the first functional data on iκbε in salmonids.

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PHYLOGENETIC ORIGINS OF THE COMPLEMENT SYSTEM IN THE ANCESTOR OF ANIMALS

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An immune revolution was likely required for the first animals to evolve multicellularity in a microbial world, yet the mechanisms they used to distinguish pathogens from microbiota remain largely unknown. Many bilaterian immune pathways have distant homologues in sponges, but not comb jellies, while others are proposed to have evolved later, including the complement system, an important front-line innate immune system that triggers phagocytosis, inflammation, and pathogen destruction. Uncertainty surrounding the branching order of non-bilaterian animals and the selection of outgroups for immune gene phylogenies, together with recurrent gene loss, creates a compound problem for inferring the immune pathways present in the first animals. Here, we present orthologs of core components of the complement system in multiple non-bilaterian lineages and identify unexpected ancient duplicates of complement system genes in early animal evolution that have been lost in humans. Furthermore, by utilizing multiple bioinformatic techniques to root phylogenetic trees, we side-step gene and pathway loss events in multiple non-bilaterian lineages to reveal that the core of the complement system predates the divergence of extant animals.

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IDENTIFICATION OF A NEW PHARYNGEAL MUCOSAL LYMPHOID ORGAN IN ZEBRAFISH AND OTHER TELEOSTS: TONSILS IN FISH?

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