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Abstract

Rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) represent key species in aquaculture and are important models for the development of fish immunology. As in mammals, the basis of teleost humoral adaptive immune response is the clonal expression by B cells of somatically diversified immunoglobulins (IG), either as membrane bound or secreted in response to infections or immunizations. The IG repertoire sequencing has started to develop both in rainbow trout and in Atlantic salmon, reflecting a growing interest for an accurate and comprehensive description of the response against common pathogens and vaccines. In this context, a unified and standardized nomenclature and classification of IG genes is needed. In addition, these species are of particular interest because their IG loci are complex due to two additional whole genome duplication (WGD), compared to tetrapods: a WGD event that occurred during early teleost evolution, and a recent WGD that is specific to salmonids. This is reflected in the identification of IGH isoloci on two chromosomes. A good quality genome assembly is now available for rainbow trout and Atlantic salmon allowing a fully annotation that provide novel information. Here, we present how an IMGT-based nomenclature, numbering and structural description can be established in the frame of the Inferred Allele Review Committee (AIRC) working group, and how it helps comparing the diversity, the structure and the dynamics of antibody repertoires between fish and mammals.

Keywords: Immunoglobulin, locus, repertoire, nomenclature, Salmonids

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O-038.

Metagenome analysis of intestinal flora in the IL-17A/F1-knockout medaka

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Abstract

In mammals, interleukin (IL)-17A and IL-17F are hallmark inflammatory cytokines, which are expressed by Th17 cells and play key roles in protection against infection and intestinal mucosal immunity. However, although fish IL-17A and IL-17F homologs named as IL-17A/F have been identified, their functional aspects, especially in intestinal mucosal immunity are still poorly understood. In this study, IL17A/F1-knock-out (IL17AF1-KO-) medaka (Oryzias latipes) was established using the genome-editing technique, CRISPR/Cas9 system, and a 7-bp deletion (-7bp) and a 11-bp addition (+11bp) were confirmed in the IL-17A/F1-KO-medaka. After establishing F3 homo KO-medaka (+11bp), we conducted bacterial infection test with Edwardsiella tarda (E381 strain) to compare the defense capability in intestine of IL-17A/F1-KO-medaka to those of wild type (WT) medaka. After 24 hours immersion in freshwater containing 2.1Å~108 CFU/ml E. tarda, the number of bacteria was higher in posterior intestine than in anterior intestine in both WT and IL-17A/F1-KO-medaka. However, after 48 hours, bacterial number in posterior intestine decreased to the same extent as in anterior intestine at the same time. Furthermore, in comparison between WT and IL-17A/F1-KO-medaka, bacterial number of E. tarda in posterior intestine of IL-17A/F1-KO-medaka increased in 24 hours compared to those of WT. In addition, the results of gene expression in intestine by real-time PCR (qPCR) showed that antimicrobial peptide genes such as G-type lysozyme and transferrin a after infection were significantly down-regulated in IL-17A/F1-KO-medaka compared to those of WT. Furthermore, we performed 16S rRNA-based metagenome analysis to compare changes in composition of intestinal bacterial flora during naïve and infection between IL-17A/F1-KO and WT medaka. As a result of α diversity analysis, under naïve condition, the diversity of bacterial flora was less in the WT medaka than in the KO medaka. After infection, although, the diversity of bacterial flora increased in both KO and WT medaka, bacterial species of WT medaka increased over twice in 24 and 48 hours after infection in comparison to those of naïve group, while there was a 1.5 times increase of bacterial species in IL-17A/F1-KO groups in 24 and 48 hours after infection. Furthermore, in weight-UniFrac analysis, it was revealed that WT and IL-17A/F1-KO group under naïve condition form different clusters. These results suggested that IL-17A/F1 induces a change in the composition of the intestinal bacterial flora in medaka.

Keywords: Interleukin 17, Japanese medaka, Antimicrobial peptide, 16S rRNA-based metagenome, Genome editing

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O-039.

Differential microbiota and immune modification in rainbow trout when facing bacterial infection

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Abstract

Abstract must have a maximum of 2700 characters including spaces. In metazoans, the epidermal surface is important to maintain homeostasis of individuals. These epidermis are colonized by bacteria that have co-evolved with the host and that form communities with a complex network of interactions, called the microbiota. Communication between microbiota and the host was made possible by developing a suitable immune system. The microbiota is involved in many crucial functions for the host such as the maturation and stimulation of innate and adaptive immunity and the defense against pathogens by avoiding their colonization. Therefore, it is essential for the host to maintain homeostasis within the microbiota and between its mucosal immune system and the microbiota to keep functionality. However, this communication between those two compounds can be disrupted by various kinds of stressors present in the organism's environment. Such disturbance of this homeostasis is called a dysbiosis and can lead to detrimental, even mortal consequences for the host. Among these stressors, we can find some diseases caused by bacterial infection such as *Aeromonas salmonicida*. This pathogen is the causative agent of furunculosis and lead to important mortality in aquaculture. In this study, we have described the microbiota from different epithelial locations (skin, gills, caudal fin) exposed to a bacterial stressor (*Aeromonas salmonicida achromogenes*) using Next Generation Sequencing (Illumina HiSeq 2500). The hypervariable region V1-V3 16S rRNA gene was

sequenced to assess taxonomical composition and structure of different epithelia 6 h and 72 h after a short bacterial bath infection (1 h). We also assessed the immune status of fish after bath infection through qPCRassays. Alpha diversity measurements (inverse Simpson and Shannon index) suggest that the different microbiotas are highly diverse but predominated by few taxa and that the bacterial infection does not affect these indices. On the other hand, beta diversity measurements showed a potential early infection through gills after 6 h,then affecting skin and caudal fin microbiotas. Negative binomial generalized linear model (nb-GLM) highlighted the increase of different opportunistic pathogens such as Aeromonas, Pseudomonas, etc 6 h after bath infection. These observations are consistent with immune assays (cytokines, humoral response, gene expressions) showing a response of the immune system after 6 h followed by a disorder in its functioning. This study suggests that furunculosis not only impair immune system in rainbow trout but also induce dysbiosis leading to the increase of opportunistic pathogens in the bacterial community.

Keywords: Aquaculture, Microbiota, pathogen infection, immune system, *Oncorhynchus mykiss*

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O-040.

TLR5M and TLR5S play opposite roles in NF-ΚB pathway in *Vibrio parahaemolyticus* flagellin stimulation in orange-spotted grouper, *Epinephelus coioides*

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Abstract

A family of Toll-like receptor (TLRs) acts as primary sensors, which detect various microbial components, activate the host innate immune response to eliminate invading pathogens. In mammals, nuclear factor (NF)-κB signaling pathway is critical to the inflammatory cytokines and effectors expression. However, in fish, the bidirectional regulation of TLRs on NF-κB pathway is poorly understood. Here, we investigated the potential molecular mechanism of orange-spotted grouper (*Epinephelus coioides*) TLR5M (*Ec*TLR5M) and TLR5S (*Ec*TLR5S) regulating the NF-κB pathway after *Vibrio parahaemolyticus* flagellin stimulation.

*Ec*TLR5M, a member of the conserved TLR protein family, is involved in specifically recognizing flagellin and activating the NF-κB pathway. After knockdown of *Ec*TLR5M in grouper spleen (GS) cell line, the phosphorylation of IκBα and the expression of downstream cytokines, such as interferon (IFN) -γ2, interleukin (IL) -6 and tumor necrosis factor (TNF) -α, were all significantly suppressed. The overexpression of *Ec*TLR5M induced not only the activation of NF-κB pathway, but also mRNA expression of *Ec*TLR5S.

*Ec*TLR5S, consisting of 17 extracellular leucine-rich repeat domains, is located in the cytoplasm and involved in flagellin recognition. Knockdown of *Ec*TLR5S enhanced the phosphorylation of IKKα/β and IκBα, promoted NF-κB p65 nuclear transport, and augmented the cytokines IFN-γ2, IL-6 and TNF-α mRNA expressions after flagellin stimulation. Consistently with these observations, over-expression of *Ec*TLR5S negatively regulated the NF-κB pathway activation. We verified that the N-terminal (aa 1-254) and C-terminal (aa 514-643) of *Ec*TLR5S are the major functional domains of negative regulation by deletion mutation.

Taken together, *Ec*TLR5M is identified as a positive regulator, activating the NF-κB signaling after flagellin recognition. And for the first time, *Ec*TLR5S is demonstrated as a negative regulator that suppresses flagellin-induced activation of NF-κB, suggesting an important role for *Ec*TLR5S in control of innate immunity homeostasis.

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Keywords: NF-κB; TLR5M; TLR5S; flagellin; cytokine

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O-041.

Bacterial outer membrane vesicles of *Aeromonas salmonicida* induce a proinflammatory immune response in vitro and in vivo

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Abstract

High mortality rates after bacterial infections cause huge annual losses for the aquaculture industry. As treatment with antibiotics is not an alternative, bacterial vaccines for intramuscular or intraperitoneal injection were developed resulting in protection but also in inflammatory granulomas and stress. Here we propose the design of a modular vaccine based on outer membrane vesicles (OMV's) of the bacterial fish pathogen *Aeromonas salmonicida* (*A. salmonicida*.). The simple preparation, the safety due to their non-replicative nature as well as the composition of natural surface exposed membrane antigens in their native confirmation are the advantages of such a vaccine design. In the present project, the innate immune response to OMV's in comparison to bacterial stimulation was characterized using a peritoneal model for rainbow trout (*Oncorhynchus mykiss*). The distribution, recruitment and kinetics of myeloid cell populations in peritoneum, blood, spleen and head kidney were compared using lineage marker specific monoclonal antibodies. Additionally, the monocyte/macrophage cell line RTS-11 was used to characterize the mRNA profile response of phagocytes to OMVs and *A. salmonicida* bacterial particles.

First results indicate similar patterns of cellular responses in vivo either by stimulation with OMVs or with bacteria in regards to cell kinetics as well as to the induction of pro-inflammatory genes.

Next steps will include engineering of recombinant *A. salmonicida*, which produce of OMVs, presenting the immunogenic G-proteins of Viral-hemorrhagic-septicemia-virus (VHSV), Infectious-hematopoietic-necrosis-virus (IHNV) and Spring-viraemia-of-carp-virus (SVCV).Those OMV's will be used to analyze the innate immune response against bacterial and viral pathogens in regard to induction of protective immune memory.

Keywords: *Aeromonas salmonicida*, outer membrane vesicles, *Oncorhynchus mykiss*, innate immune response, vaccine

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O-042.

Impact of sea lice (*Caligus rogercresseyi*) infection levels on skin transcriptome in Atlantic salmon (*Salmo salar*)

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