

O-048.**The transcriptome reveals the molecular mechanisms underlying hemocytes differentiation in the oyster**Yang, Zhang, Ziniu Yu[#].

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Abstract

Hemocytes are considered to be the central component of internal immune defense system in many invertebrates including oysters. Morphological and functional classification demonstrate that the granulocytes rather than hyalinocytes are the main immune executor since the former exhibited stronger capacities either in phagocytosis or ROS production. However, the molecular mechanism underlying granulocytes cytogenesis is still unclear. In this study, two main cell types, granulocytes and hyalinocytes were sorted and further studied based on the flow cytometry from hemocytes of the Pacific oyster *Crassostrea gigas*. Transcriptomic analyses from four biological replications revealed the 175 of core differentially expressed genes (DEGs) were significantly high expressed in granulocytes when compared with hyalinocytes. Moreover, these core DEGs were also highly expressed specifically in hemocytes rather than other tissues. The pathway network analysis revealed that phagocytes were highly activated together with actin cytoskeleton regulation, phagosome, MAPK signaling, lysosome and others. Meanwhile, the *cdc42*, as one of the key hub-genes connected multiple pathways, was also confirmed to be involved in regulation of phagocytosis and ROS production by treatment of pharmacological inhibitors. Finally, the RNAi of the key transcriptional factors show that FOS are responsible for transcriptional activation of many phagocytosis and ROS production-related genes, and typical FOS-binding sites (AP-1) were also found in proximal promoter of these genes, strongly suggesting the regulatory role of FOS in the cytogenesis of granulocytes. In a word, these results provide a novel insights into gene regulatory network underlying hemocytes differentiation of oyster.

Keywords: Oyster, hemocytes differentiation, granulocytes, phagocytosis, FOS

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Abstract

Early-life adversity has an important influence on immune responses during the lifespan development. In mammals, early-life stress was related to long-term consequences on immune functions. In fish, the exposure to a brief handling stressor or xenobiotics during early-life influenced the stress sensitivity and immunity in adulthood. So, it seems that challenges of various natures (e.g. social, chemical, physical or biological) during early-life can impact or shape an individual physical and mental's health across the lifespan in fish. Bacterial infections during early life (embryonic and/or larval) stages are very common in fish, long before the attainment of immunocompetence, when the immune system is still developing. Zebrafish larvae only possess a fully developed immune system by 4–6 weeks post fertilization. During the first weeks of life, zebrafish larvae

simply rely on efficient components of the innate immune system, most of which are already functional at the first day of embryogenesis. In this study, we aimed to evaluate the effects of different bacterial challenges during early development on zebrafish and on its immune system later in its life, notably for genes involved in immune responses against pathogenic infection. Thus, four histories of infection with a virulent strain of *Aeromonas salmonicida achromogenes* were tested in the first month post-hatching: control group without any infection, zebrafish exposed to an early infection at 15 days post-hatching (dph), zebrafish chronically exposed to bacteria, from 15 to 32 dph, and zebrafish exposed later at 32 dph. Then, all groups were maintained in tanks and exposed to the same pathogen at 58 dph. Fish were sampled before infection, and at 6h and 24h post-infection. The analysis of immune gene expression in early life stages of fish revealed that the age of first infection influenced the responses (level of expression and timing) of immune system including bacterial lysis, opsonization or phagocytosis (*C3a component*, *myeloperoxidase*), inflammatory processes (*il-6* and *cox 2*) and adaptive immunity (*cd4*, *rag 1* and *tcrα*). In addition, the exposure to the pathogen in juvenile stage (58 dph) induced differential innate immune (including *c3a*, *lysozyme*, *mpo*, *transferrin*) and inflammatory responses (*il-6*, *cox2*, *tgfb*) depending on infectious history during the early life. These results suggest that the infection at larval stage, when adaptive responses are not yet effective, will influence immune pathways later in life and offer new perspectives to study the molecular modifications affecting the memory of immune system such as epigenetic mechanisms.

Immune responses to bacterial infection in zebrafish juveniles with different early-life infectious histories

Keywords: Zebrafish; bacterial challenge; early infection; long-term effects; molecular analysis.

[#] Corresponding author.E-mail address: valerie.cornet@unamur.be (V. Cornet).^{*} These authors have contributed equally to this work.**O-050.****Distribution of different populations of t cells and b cells in the interbranchial lymphoid tissue (ILT) of salmon**O.M. Løken¹, H. Bjørgen¹, I. Hordvik², E.O. Koppang^{1, #}.¹ Section of Anatomy and Pathology, Veterinary Faculty, The Norwegian University of Life Sciences, Oslo, Norway² Institute of Biology, University of Bergen, Bergen, Norway**Abstract**

The interbranchial lymphoid tissue of Atlantic salmon (*Salmo salar*) consists of T cells embedded in a matrix of reticulated epithelial cells. The amount of B cells is very limited. Here we use *in situ* hybridization probes to detect transcripts of different specific T and B cell genes. We show that the structure is rich in α/β T cells but less so in γ/δ T cells. In the thymus, the distribution of such cells is localized in distinct zones, but that is not the case in the ILT. The constitutively expressed cytokine CCL19, which is important in T cell zones in mammalian lymphoid tissues and attracts naïve T cells (but also other leukocytes), is evenly distributed in the ILT but restricted to cortical regions in the thymus. IgT expressing cells in the ILT are less common than IgM-expressing cells. Control tissues included skin and head kidney. The use of *in situ* hybridization has allowed us to reveal specific anatomical features of the ILT and the thymus regarding leukocyte cell distribution. The experiments have shown that even though the ILT and the thymus share some common features, the anatomical organization of the tissues are profoundly different.

Keywords: B cell; gills; ILT; T cell; thymus

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