

O-018.**Identification of primordial organized lymphoid structure in the spleen of teleost fish**

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Abstract

Induction of immune responses in higher vertebrate species occur within organized lymphoid structures (e.g. lymph nodes, Peyer's patches). These structures probably emerged throughout evolutionary time with the goal to maximize encounters between antigens, antigens-presenting cells and lymphocytes. The current dogma states that Teleost fish lack such structures and thus, it is ill-understood how adaptive immune responses develop in these species. This knowledge in fish has been held back for decades due to the lack of reliable antibody reagents with the capacity to recognize the different subsets of the fish leukocytes involved in the induction of adaptive immune responses.

Over the last few years our laboratory have produced several antibody reagents that recognize different subsets of B and T cells. These antibodies have enabled us to start addressing the mechanism and cells involved in the generation of adaptive immune responses in fish. To understand how immune responses are induced in teleost lymphoid organs, we infected fish with *Ichthyophthirius multifiliis*, a parasite that induces both systemic and mucosal antibody responses in Rainbow Trout. Immune responses were followed with a panel of anti-leukocyte antibodies using flow cytometry as well as immunofluorescence, and 3D confocal microscopy, which enabled the analysis of the kinetics and spatial organization of proliferative and resting B and T lymphocytes respectively. Overall, our results identified the spleen as the major site for CD4+ T and IgM+ B cell proliferation in systemic lymphoid organs upon infection. The proliferating splenic IgM+ B cells were frequently observed as clusters in the vicinity of melano-macrophage centers. Moreover, in these areas we observed aggregates of B and T lymphocytes with a loose organized structure reminiscent of the cellular architecture frequently associated with tertiary lymphoid organs. Laser dissection microdissection of these areas has enabled us to start evaluating both the transcriptome and immunoglobulin repertoire that characterize these structures upon infection. In conclusion, these data offer important clues regarding the cellular structures and mechanisms by which adaptive immune responses develop in teleosts, and suggest the existence of primordial semi-organized lymphoid tissue in the spleen in which such responses are induced. **Keywords:** IgM+ B cell, CD4+ T cell, adaptive immune response, organized lymphoid structure, semi-organized lymphoid structure

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O-019.**Immunomodulatory role of secreted IgD in rainbow trout**

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Abstract

In mammals, IgM and IgD are co-expressed on the surface of naive B cells, which, upon antigen binding, down regulate IgD expression, accounting for the gradual disappearance of IgD from the cell surface of activated cells that goes along with somatic hypermutation and class-switch DNA recombination to diversify the Ig gene repertoire. Nevertheless, IgD seems to play a relevant role in the human upper respiratory tract where IgD-secreting cells are found. In humans, this secreted IgD has been shown to play a role in immune surveillance through the activation of basophils and mast cells. In fish, IgD+IgM- B cells have been identified in rainbow trout gills and catfish blood. In both cases, these cells have been shown to have a plasmablast phenotype and are thought to be responsible for the production of secreted IgD found in the serum of these species. However, the immune role of this secreted IgD in teleost remains uncertain. In this study, we demonstrate an immunomodulatory role for secreted IgD. Stimulation of kidney leukocytes with IgD purified from rainbow trout serum provoked a strong immunomodulatory effect, in which the transcription of many immune genes was significantly up-regulated, including pro- and anti-inflammatory cytokines and complement factors. Flow cytometry was used to characterize the cell population which was binding and responding to secreted IgD among head kidney leukocytes. Finally, experiments aimed at characterizing the interplay between secreted IgD and microbiota were also undertaken. Our results demonstrate that as in mammals, secreted IgD has an immunomodulatory role regardless of its antigen specificity.

Keywords: Rainbow trout, secreted IgD, kidney leukocytes, immunomodulation, microbiota.

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O-020.**Rainbow trout CD38 defines a subset of B cells in rainbow trout**

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Abstract

CD38 is a member of the ribosyl cyclase family. It is a multifunctional molecule that functions both as a transmembrane signaling receptor and as an ectoenzyme with important roles in cell adhesion, calcium regulation and signal transduction. In mammals, although expressed in different leukocyte subsets, within the B cell lineage, CD38 is usually considered an activation marker. In humans, CD38 is transiently expressed on early lymphocyte precursors, is lost on mature lymphocytes and is consistently expressed on terminally differentiated plasma cells. In the present work, we have identified a homologue of CD38 in rainbow trout (*Oncorhynchus mykiss*). Using a newly developed monoclonal antibody against this CD38 molecule, the presence of CD38+ populations among IgM+B cells and IgM- leukocytes has been investigated in different rainbow trout lymphoid and mucosal tissues through flow cytometry and immunofluorescence techniques. Moreover, after cell sorting of the different populations identified, the molecular expression profile of each subset has also been determined. Finally, the capacity of different cytokine combinations to regulate the percentage of CD38+ populations has also been established. This study contributes to further understanding B cell differentiation processes in teleost, through the identification of novel cell subsets among B cells.

Keywords: Rainbow trout, CD38, B cells, plasma cells, IgM

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