Fish & Shellfish Immunology 34 (2013) 1635–1691

Contents lists available at SciVerse ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Oral Abstracts from the first conference of the International Society of Fish and Shellfish Immunology

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http://dx.doi.org/10.1016/j.fsi.2013.03.006

gmDB has a typical cysteine bridge pattern and α/β fold with an α and β1β2β3 sheets, in common with other β-defensins. Expression of the gmDB gene was developmentally regulated, since its transcripts were detectable from the golden-eye stage onwards and restricted to swim bladder and retina. Similarly, gmDB transcripts were found in some tissues of juvenile fish, including skin, swim bladder, peritoneum wall, head kidney excretory kidney and ovary. In situ hybridisation revealed that gmDB was constitutively expressed specifically by cells located in the submucosa of the swim bladder and in developing oocytes. Transcription of gmDB was up-regulated up to 25-fold in head kidney in response to antigenic challenge with *Vibrio anguillarum*. Recombinant gmDB displayed antibacterial activity mainly towards Gram-(+) bacteria, with minimal inhibitory concentrations of 125 μg/ml and 250 μg/ml against *Planococcus citreus* and *Micrococcus luteus*, respectively. Taken together, our data indicate that β-defensins may play an important role in the innate immune response of Atlantic cod.

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O-347.

Antiviral activity of Mx proteins from gilthead seabream (*Sparus aurata*) against lymphocystis disease virus (LCDV)

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Abstract
Gilthead seabream (*Sparus aurata*), one of the most important fish species in the Mediterranean Aquaculture, displays a high natural resistance to viral diseases. Until now, the main viral disease affecting gilthead seabream is the lymphocystis disease (LCD), caused by the lymphocystis disease virus (LCDV). LCDV is a DNA virus belonging to the *Iridoviridae* family. The predominant symptom of the disease is the presence of visible papiloma-like skin lesions. Although LCD is a self-limiting infection, it may cause high morbidity and, therefore, important economic losses for the industry.
One of the main components of the immune system to fight viral infections is the interferon-mediated response. This cytokine is secreted by infected cells to promote an antiviral state in neighboring cells through the induction of several proteins, some of them with direct antiviral activity. One of these interferon-induced proteins with direct antiviral activity is the Mx protein. Our group has identified three Mx proteins in gilthead seabream, named SauMx1, SauMx2 and SauMx3. To evaluate their antiviral activity, three *in vitro* experimental systems were established, consisting of CHSE-214 cells stably expressing each Mx protein separately. In this study, the activity of these three Mx proteins has been tested against LCDV. Results showed that SauMx1 and SauMx2 have antiviral activity against this virus, as viral titers were reduced 96 and 856 fold for SauMx1 and SauMx2, respectively. In contrast, the presence of SauMx3 did not inhibit LCDV replication in the cells. As far as we know, this is the first description of a fish Mx protein with antiviral activity against LCDV. Thus, a deeper knowledge on this antiviral activity, and on the interactions taking place between gilthead seabream Mx proteins and LCDV, could help to design strategies against LCD.

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O-117.

Anatomy of the interbranchial lymphoid tissue (ILT) in salmonid fish

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Abstract
It has long been postulated that teleosts lack organized mucosa-associated lymphoid tissue (MALT), but with the discovery of the interbrancheal lymphoid tissue (ILT) in salmonid gills, this assumption may be reconsidered. The hallmark of organized MALTs is the presence of lymphoid follicles, consisting of naïve B cells flanked by T cell-rich areas. The ILT consists predominantly of T cells embedded in a meshwork of epithelial cells and is devoid of blood vessels and has thus limited resemblance with previously described lymphoid tissues. However, populations of B cells are also present in the ILT, and we have initiated investigations of their nature. Further, we have started investigations of the stromal cells forming the meshwork of the ILT in comparison to other lymphoid salmonid tissues, aiming at further elucidating the nature of the structure. The latest findings in our characterization efforts of the ILT including ultrastructural and transcriptional data will be presented together with the initial investigations leading to its discovery.

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O-468.

Characterization of the salmonid cathelicidins and of their biological activities

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Abstract
Cathelicidins are a family of cationic antimicrobial peptides (AMPs) and are an important component of innate immune response.
Two members of this family have recently been identified in salmonids and other fish. Analysis of the *cath-1* and *cath-2* salmonid cathelicidin gene sequences showed a protein organization with a characteristic conserved cathelin-like N-terminal domain and a varied glycine/serine–rich C-terminal domains corresponding to the active peptides.
In this study we characterized the antimicrobial activity spectrum, the mode of action and tissue expression of salmon cathelicidins. Different peptide fragments representing specific C-terminal regions of CATH-1 and/or CATH-2 of rainbow trout (*Onchorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), grayling (*Thymallus thymallus*) and brown trout (*Salmo trutta fario*) have been chemically synthesized and their antimicrobial activity evaluated against standard bacterial strains and some fish pathogens.
Most peptides showed a medium-dependent antimicrobial activity with MIC values ranging from 4 μM and 64 μM. Killing kinetics, membrane permeabilization assays and hemolytic assays indicated that these peptides rapidly kill bacteria by permeabilization of their cell membranes and at the same time show very low toxicity against erythrocytes. To detect CATH-1 in trout tissues and to study its processing a polyclonal antibody was raised against a complete recombinant CATH-1 protein derived from O. mykiss spleen cDNA. Western blot analysis revealed that CATH-1 protein is express in spleen and head kidney tissues of trout.
To evaluate gene expression and tissue distribution of CATH-1 and CATH-2, an *in vivo* experiment was performed infecting *O. mykiss* samples with the salmon pathogen *Yersinia ruckeri* and tissues have been collected at different times and analyzed by real-time RT-PCR. Data evidenced a high induction of the expression 24 hours post-infection in spleen (average induction i.e. 10- and 180-fold for CATH-1 and CATH-2 respectively), head-kidney and in intestine 48 hours after the challenge.
Results will contribute to a comparative understanding of the functions of cathelicidins in the vertebrates.

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