

O-062.**Vaccination and immune response of the pituitary in fish**

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Abstract

Although it is known that pituitary hormones can have a direct or indirect influence stimulating or suppressing the immune responses, whether there is a local immune response in this tissue or what is the effect of the immune stimulus on the pituitary function in fish has not received specific attention. In order to understand the immuno-endocrine interaction at the pituitary level, particularly the Hypothalamus-Pituitary-Interrenal axis, different experiments were carried out in rainbow trout and gilthead seabream using both *in vitro* and an *in vivo* approaches. Pituitaries of rainbow trout *Oncorhynchus mykiss* were cultured *in vitro*, incubated for 3h with *Vibrio anguillarum* bacterin, bacterin plus CRH, cortisol, human recombinant IL1 β , or spleen medium, and then genes involved in pro-inflammation (*il1 β* , *il8*, *tnf α 1*, *ifn γ*), anti-inflammation (*tgf β 1b*, *il10*), or innate immune modulation (*mhclla*, *c3*, *mif*) were tested. Data showed that, incubation with bacterin alone and bacterin plus recombinant IL1 β or CRH, as well as medium from bacterin-treated spleen caused significant up-regulation of pro-inflammatory genes, while down-regulated the anti-inflammatory gene *tgf β 1b*. Besides, recombinant IL1 β plus bacterin or alone caused raise of *mhclla* and *tnfa*, respectively. A second experiment assessed the response of seabream vaccinated by means of an injection with *Lactococcus garveiae* and immune gene modulation was determined after 1h, 6h and 24 hours looking at the expression of the proinflammatory genes *il1 β* , *tnfa* and *cox2*, the anti-inflammatory genes *tgf β 1b*, *il10*, and the innate genes *lys* and *c3* corresponding to lysozyme and complement proteins. The immune genes *il1 β* , *cox2* and *lys*, showed a strong expression in the pituitary tissue after injection vaccination, notably *il1 β* which showed more than 10 fold raise, thus indicating both a high sensitivity to the vaccine and the onset of a robust immune response in the pituitary at *in vivo* level. The overall results indicate that pituitary shows a relevant local immune gene equipment, and also the potential of fish pituitary to develop both innate and adaptive immune responses.

Keywords: Vaccination, pituitary, trout, seabream, immune-response

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O-063.**The evolutionary puzzle of IgT genes in antarctic fishes**

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Abstract

The Perciform suborder Notothenioidei represents the major component of the Antarctic fish fauna, comprising five Antarctic families, Channichthyidae, Bathydraconidae, Artedidraconidae, Nototheniidae, and Harpagiferidae, and three non-Antarctic families, Bovichtidae, Pseudaphritidae,

and Eleginopidae. Notothenioids have evolved a variety of peculiar anatomical, physiological and biochemical features to adapt to the extremely cold Antarctic environment, providing an extraordinary model system to identify gene changes and investigate their importance for adaptive evolution. We have previously isolated and characterized IgT heavy chain constant region gene of the Antarctic teleost *Trematomus bernacchii* (family Nototheniidae), discovering that *T. bernacchii* IgT lack almost the entire heavy chain second constant domain, retaining only a few of amino acid residues. By analyzing cDNA sequences encoding IgT heavy chain three differently sized IgT transcript variants were identified, named Long, Short, and Shortest, 51-bp, 33-bp, 42-bp long, respectively. The aim of the present study is to provide a framework for understanding the loss of the CH2 domain through the notothenioid phylogeny. To this end, we isolated and characterized IgT genes from other species belonging to families Nototheniidae, Bathydraconidae and Artedidraconidae. In all cases the remnant CH2 exon preserved the donor and acceptor splicing sites to be correctly spliced into the mature transcript, giving rise to different cDNA variants: 24-51 bp long (8-17 aa) according to the species analyzed. Moreover, one representative each of the two non-Antarctic families was included in our studies for comparison: *Eleginops maclovinus* (family Eleginopidae), and *Bovichtus diacanthus*, (family Bovichtidae). Both diverged early from the main notothenioid lineage, before a severe decrease in water temperature and climatic and geographic isolation of Antarctica. A comparative analysis at genomic level has highlighted that the remnant CH2 exon is shared by all Antarctic fish families analyzed in the present work. Amazingly, the loss of most CH2 is shared also by *E. maclovinus* but not by *B. diacanthus*. These results may help shed light on the evolutionary processes that underlie the origins of such gene modifications.

Keywords: IgT, Antarctic teleost, evolution, exon remnant, genome modifications

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O-064.**Head kidney- and trunk kidney-derived macrophages differentially respond to stress and cortisol**

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Abstract

In teleosts, hematopoiesis, including myelopoiesis, is maintained in the head (HK) and trunk kidney (TK). Moreover, HK, but not TK, functions as endocrine organ where cortisol is produced. Therefore, it is postulated that macrophages from both sources differentiate in different hormonal microenvironment where HK-born macrophages are under direct paracrine action of cortisol. Interestingly, in mammals cortisol has been described as one of the factors inducing alternative anti-inflammatory M2 polarization of macrophages.

For example, in RAW 264.7 macrophages it decreased gene expression of pro-inflammatory mediators (e.g. IL-1b and inos). Here we aim to compare effect of stress (*in vivo*) and cortisol (*in vitro*) on the polarization of carp macrophages derived from HK and TK. Carp macrophages from both sources were separated and *in vitro* treated (6 h) with cortisol (CORT, 1 μ M), lipopolysaccharide (LPS, 30 μ g/ml) or with CORT+LPS. *In vivo* fish were stressed (restraint stress for 24h) or left undisturbed. Macrophages from both animal groups were isolated and *ex vivo* treated with LPS (6h). Gene expression of several markers of classical M1 (iNOS, IL-1b, IL-12p35, CXCL8 and CXCb) and alternative M2 (arginase, IL-10, MMP-9) macrophage polarization and expression of genes encoding cortisol receptors (GR1-2) were measured. We found that CORT *in vitro* in LPS-treated HK and TK macrophages down-regulated gene expression of M1 markers: *il-1b*, *cxcl8* and *cxcb1* while in TK-derived macrophages CORT induced down-

regulation of the gene expression of both M1 (il-12p35) and M2 (il-10) markers and both gr genes. Upon stress, freshly isolated HK macrophages had higher gene expression of M2 markers (arginase 2, IL-10 and MMP-9) than cells from control fish. Interestingly, in TK macrophages from stressed animals, next to up-regulation of IL-10 and MMP-9 genes, stress induced down-regulation of IL-1b and CXC chemokines.

Moreover, LPS-treated HK macrophages from stressed fish down-regulated il-12p35, cxcl8_l2 and cxc1 gene expression and up-regulated gr2 expression whereas at the same conditions TK-macrophages upregulated gene expression of CXC chemokines and down-regulated expression of arginase 2. All together our data suggest that, however in fish macrophages cortisol and stress induce alternative M2 polarization this can be differentially manifested in HK- and TK-derived cells as in HK macrophages stress up-regulates M2 markers while in TK cells it up-regulates M2 markers and at the same time down-regulates M1 markers.

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Keywords: macrophage polarization, stress, cortisol, carp

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O-065.

Detection of interleukin (IL)-22 protein expression in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

IL-22 is a critical cytokine which is involved in modulating tissue responses during inflammation, and is produced mainly by T cells and innate leucocytes. In mammals, IL-22 is a key component in mucosal defences, tissue repair, epithelial cell survival and proliferation. In teleosts, IL-22 has been cloned and studied in several species, and the transcript is highly expressed in mucosal tissues and induced by pathogen associated molecular patterns (PAMPs), suggesting IL-22 also functions as an important component of the innate immune response in fish. To investigate these immune responses further, we have validated and characterised two monoclonal antibodies (mAbs) which were raised against two different peptide immunogens of salmonid IL-22. Our results showed that both mAbs specifically react to their own peptide immunogens and recombinant IL-22, and are able to detect the induction of native protein expression after stimulation. In flow cytometry, an increase in IL-22 positive cells was detected after stimulation *in vitro* with cytokines and PAMPs and *in vivo* after bacterial challenge. The immunohistochemistry results showed that IL-22 is highly upregulated in the gills after challenge, both in cells within the gill filaments and in the interbranchial lymphoid tissue (ILT). Such results suggest IL-22 may have a role in triggering local antimicrobial defences in fish that may facilitate efficient microbial clearance. Hence monitoring IL-22 producing cells/protein secretion may provide an alternative mean to assess the effectiveness of mucosal vaccines.

Keywords: Rainbow trout, cytokine, IL-22, protein expression, mucosal immunity

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O-066.

Structure of grass carp interleukin-2 provides insights into the evolution of four α -helical cytokine family

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Abstract

Interleukin (IL) -2 is a member of four α -helical cytokine family which also comprises IL-4, IL-7, IL-9, IL-15 and IL-21. It is primarily expressed in activated CD4+ and CD8+ lymphocytes and plays a crucial role in mediating adaptive immune response. In this study, the grass carp (*Ctenopharyngodon idella*) IL-2 (CiIL-2) was sequenced and its crystal structure determined. The open reading frame of the CiIL-2 gene is 426 bp, that translates into a protein of 142 amino acids, with a predicted signal peptide of 20 aa. Analysis of the crystal structure revealed that the CiIL-2 displayed a classic cytokine structure consisting of four helical bundles. Compared with the human counterpart, the CiIL-2 has a remarkably straight second helix with a significant conformational change in the region for receptor binding. Besides, the key hydrophobic amino acids which interact with the receptors in mammals are not conserved in CiIL-2. The CiIL-2 is predominantly expressed in lymphocyte-rich tissues such as spleen, kidney and thymus and is able to enhance the proliferation of primary leucocytes and the expression of STAT5 and interferon gamma. Our results suggest that IL-2 could have undergone considerable structural changes in order to facilitate interaction with its receptors during evolution.

Keywords: Grass carp (*Ctenopharyngodon idella*), fish, interleukin-2, crystal structure, evolution

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O-067.

Immunological effects of functional feeds on *Penaeus monodon* naturally infected with gill-associated virus

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Abstract

Functional feeds are becoming increasingly common to help prevent and control disease losses in marine shrimp farming. Functional feeds contain additional compounds beyond the basic nutritional requirements of the animal that result in improved health and/or growth. Common additives include probiotics, prebiotics, immunostimulants, vitamins and nucleotides. In this study we assessed three functional feeds containing either B-glucan, poly-hydroxybutyrate or a marine microbial floc ingredient on their potential immunostimulatory effect on *Penaeus monodon* with pre-existing gill-associated virus (GAV) infections. Groups of *P. monodon* (mean weight of 14 g) were fed one of the functional feeds or a basal diet for two weeks. Pre-existing GAV infection loads were determined by collecting pleopod tissue from each individual on Day 0 and using RT-qPCR to quantify GAV titre. Prevalence of pre-existing GAV infections was 83% with