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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 IL1β, or spleen medium, and then genes involved in pro-inflammation (*il1β, il8, tnfα1, ifnγ*), anti-inflammation (*tgfβ1b, il10*), or innate immune modulation (*mhcIIa, c3, mif*) were tested. Data showed that, incubation with bacterin alone and bacterin plus recombinant IL1β 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 *tgfβ1b*. Besides, recombinant IL1β plus bacterin or alone caused raise of *mhcIIa* 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 *il1β*, *tnfa* and *cox2,* the anti-inflammatory *genes tgfβ1b, il10*, and the innate genes *lys* and *c3* corresponding to lysozyme and complement proteins. The immune genes *il1β*, *cox2* and *lys*, showed a strong expression in the pituitary tissue after injection vaccination, notably *il1β* 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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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, Channichthydae, 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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Head kidney- and trunk kidney-derived macrophages differentially respond to stress and corisol

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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 μM), lipopolysaccharide (LPS, 30 μg/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-