

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 cxcb1 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