

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

a mean infection load of $1.25 \cdot 10^6 \pm 3.12 \cdot 10^5$ GAV copies μg^{-1} TNA. After 14 days of feeding the experimental diets, eight shrimp per diet were sampled and their GAV infection load was quantified to determine the relative change. GAV infection loads increased over the 14 days, however, shrimp fed the three functional feeds, B-glucan, PHB or microbial floc, on average had 10-fold lower GAV infection compared to shrimp fed the basal diet. Although the functional feeds did not clear pre-existing infections, the results suggests they may have increased the immune capacity of shrimp to better control GAV proliferation compared with the standard diet. The impact of these functional feeds was further explored by measuring several immune parameters from haemolymph samples collected from the same eight prawns that GAV titre was quantified, including total haemocyte counts, phenoloxidase activity and the production of reactive oxidative species and antioxidants.

Keywords: Functional feeds, immunostimulant, GAV, immune response, shrimp

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O-068.

Antibody repertoire and kinetics in atlantic salmon following vaccination and challenge with Salmonid alpha virus

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Abstract

Immunoglobulin M (IgM) is important in protection against disease in Atlantic salmon (*Salmo salar*). For many diseases, specific antibody response may be good despite poor disease protection, and vice versa. Non-specific antibodies (NAB), broadly cross-reactive antibodies with low affinity, are typically abundant in the blood of Atlantic salmon, but their role in disease protection is poorly understood.

In this study, we have examined the antibody repertoire and the immune gene expression of Atlantic salmon following vaccination and experimental infection. Parr was vaccinated with an commercial multivalent vaccine that included inactivated Salmonid alpha virus (SAV), and three weeks after vaccination the fish was transferred to sea water and infected with SAV subtype 3 in a shedder-cohabitant model. Blood plasma and samples from lymphoid tissue and heart were collected at several time-points pre -and post-vaccination and post-infection.

Multiplex antibody assays of blood plasma from vaccinated fish showed an increase of specific antibodies to antigenic components of the vaccine after 6 - 9 weeks. In parallel, titers of non-specific antibodies increased in plasma of vaccinated fish, showing an earlier onset of increase than specific antibodies. The presence of non-specific antibodies is detected by the recognition of a synthetic hapten-carrier complex. In contrast to vaccinated fish, titers of non-specific antibodies in plasma of control (saline-injected) fish first increased after challenge with SAV. Based on these findings, high-throughput immunoglobulin sequencing (IgSeq) of the variable (antigen-binding) region of the B cell antibody receptor will be performed. With this technology, we can study the presence of shared (present in multiple individuals) and unique (present only in one individual) B-cell clonotypes in vaccinated and control fish before and after challenge. Results from microarray transcriptome analysis and RT-qPCR of selected immune genes will also be presented.

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Keywords: Vaccination, Atlantic salmon, Salmonid alpha virus, Antibody-repertoire

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O-069.

Characterization of ten CCL20-like cc chemokines in rainbow trout (*Oncorhynchus mykiss*): Sequence and expression analysis

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Abstract

Mammalian CCL20, or macrophage inflammatory protein-3 α , can function as a homeostatic and inflammatory chemokine. In relation to the latter, it is responsible for the chemoattraction of lymphocytes and dendritic cells to mucosal immune sites under inflammatory and pathological conditions. CK1, CK8A and CK8B are rainbow trout (*Oncorhynchus mykiss*) CC chemokines that were reported previously to be phylogenetically related to mammalian CCL20. In the current study, an additional seven CCL20-like paralogs in rainbow trout are reported, that are divided into three sub-groups (by phylogenetic tree, homology and synteny analysis) in agreement with past studies in fish. They have been designated here as: CCL20L1a (also referred to as CK1), CCL20L1b1, CCL20L1b2, CCL20L2a (CK8A), CCL20L2b (CK8B), CCL20L3a, CCL20L3b1, CCL20L3b2, CCL20L3b3 and CCL20L3b4. Like mammalian CCL20, rainbow trout CCL20-like molecules possess a high positive net charge with a pI of 9.34-10.16, that is reported to be important for antimicrobial activity. Rainbow trout CCL20-like paralogs are differentially expressed and in general highly expressed in mucosal tissues, such as gills, intestine and thymus. The expression levels of rainbow trout CCL20-like paralogs are increased during development and following PAMP/cytokine stimulation. For example, in RTS-11 cells CCL20L3b1 and CCL20L3b2 are highly up-regulated by LPS, poly I:C, recombinant(r) IFN α and rIL-1 β . Trout CCL20-like paralogs are also increased after *Yersinia ruckeri* infection or poly I:C stimulation *in vivo*, with CCL20L3b1 and CCL20L3b2 again highly up-regulated. Overall, this is the first report of the complete CCL20 chemokine subfamily in rainbow trout, and the analysis of their expression and modulation *in vitro* and *in vivo*. These results suggest that teleosts possess divergent CCL20-like molecules that may have important roles in mucosal immunity.

Keywords: Rainbow trout, CCL20-like chemokine, characterisation, expression, mucosal immune response

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O-070.

Temperature drives the immune response in atlantic salmon infected with sea lice: Novel insights through transcriptome sequencing analyses

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Abstract

The sea louse *Caligus rogercresseyi* is a copepod species responsible for the most relevant parasitic infections in the Chilean salmon industry. This ectoparasite causes immunosuppression and stressful conditions in farmed fishes, which led to the activation of key elements of host's immune system. However, how these host-parasite interactions are being impacted by environmental conditions such as temperature variations is