P-001.

Molecular characterization of a new fish specific chemokine CXCL_F6 in large yellow croaker (*Larimichthys crocea*) and its role in inflammatory response

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Abstract
Chemokines are a superfamily of structurally related chemotactic cytokines exerting significant roles in regulating cell migration and activation. Currently, five subgroups of fish specific CXC chemokines, named CXCL_F1-CXCL_F5, have been identified in teleost fish. However, understanding of the functions of these fish specific CXC chemokines is still limited. Here, a new member of fish specific CXC chemokines, LcCXCL_F6, was cloned from large yellow croaker *Larimichthys crocea*. Its open reading frame (ORF) is 369 nucleotides long, encoding a peptide of 122 amino acids (aa). The deduced *Lc*CXCL_F6 protein contains a 19-aa signal peptide and a 103-aa mature polypeptide, which has four conserved cysteine residues (C28, C30, C56, and C72), as found in other known CXC chemokines. Phylogenetic analysis showed *Lc*CXCL_F6 formed a separate clade with sequences from other fish species, tentatively named CXCL_F6, distinct from the clades formed by fish CXCL_F1-5 and mammalian CXC chemokines. The *Lc*CXCL_F6 transcripts were constitutively expressed in all examined tissues and significantly up-regulated in the spleen and head kidney tissues by poly (I:C) and *Vibrio alginolyticus*. Its transcripts were also detected in primary head kidney leukocytes (HKLs), peripheral blood leucocytes (PBLs), and large yellow croaker head kidney (LYCK) cell line, and significantly up-regulated by poly(I:C), lipopolysaccharide (LPS), and peptidoglycan (PGN) in HKLs. Recombinant *Lc*CXCL_F6 protein (r*Lc*CXCL_F6) could not only chemotactically attract monocytes/macrophages and lymphocytes from PBLs, but also enhance NO release and expression of proinflammatory cytokines (TNF-α, IL-1β, and CXCL8) in monocytes/macrophages. These results indicate that *Lc*CXCL_F6 plays a role in mediating the inflammatory response.
keywords: CXC chemokine, CXCL_F6, Chemotaxis, Large yellow croaker (*Larimichthys crocea*), inflammatory response

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P-002.

Molecular markers associated with antigen presentation process in Atlantic salmon during outbreaks of *Piscirickettsia salmonis* at sea farming centers

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Abstract
Chile is one of the main producers of Atlantic salmon (*Salmo salar*) in the world, with a currently marine culture biomass close to 350,000 tons. This biomass suffers mortalities close to 3,500 tons per month, being the infectious diseases the second primary cause of this problem (19.6%). The major agent of this mortality is the bacteria *Piscirickettsia salmonis* (67.9% of cases). *P. salmonis* is an intracellular facultative pathogen that generates systemic infections evading the immune response of the host by infection of macrophages and avoiding the respiratory burst. To control this pathogen, Chilean aquaculture has used antibiotics, immunostimulants and vaccines. However, no strategy has given optimal results. In this context, more data of immunological parameters in field conditions are necessary for a more accurate characterization of the real state of the fish when is facing a *P. salmonis* infection. In this work, we have focused on quantifying molecular markers associated with the process of antigen presentation, which is crucial to achieve coordination between innate and adaptive immune response. For this, we evaluated the gene expression of interferon gamma (ifng), transforming growth factor beta (tgfb) and interleukins (il-10, il-12 and il-15); markers of cell lineage (cd83 and cd80/86); major histocompatibility complex I and II (mhci and mhcii); T cell receptor alpha (tcra); immunoglobulin M (igm); and annexin1 (anxa1) by qPCR from spleen of *S. salar* at two sea farm centers (Puelche and Punta Islotes). Puelche reported two outbreaks of *P. salmonis*, while Punta Islotes didn't report any fish infected with the pathogen during the sampling time. Gene expression results showed that fish from Puelche increased the gene expression of ifng, tgfb, cd83, cd80/86, mhcii, il-10, il-12, igm and anxa1 at different sampling points. On the other hand, fish from Punta Islotes showed an increase of the gene expression of il-10 and cd80/86, mhcii and il-12. Finally, the correlation of data showed a proportional detection between markers of the same sea farm center and inversely proportional between centers with *P. salmonis* (Puelche) and without *P. salmonis* (Punta Islotes). This work was funded by the Program for Sanitary Management in Aquaculture of the Ministry of Economy, Development and Tourism of Chile (FIE-2015-V014 201708070149). BML is a fellow of Advanced Human Capital Formation of CONICYT, Chile (21151176).
keywords: *Salmo salar*, Chile, gene expression, spleen, Piscirickettsiosis

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P-003.

Evaluation of alpha-lipoic acid anti-inflammatory properties using zebrafish as in vivo model

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Abstract
Chronic diseases remain the primary root cause of death and disability worldwide. It is now well established that several agents (aging, oxidative stress, iron overload, etc,) induce inflammation and dysregulate inflammatory pathways, which lead to the development of chronic diseases. Acute inflammation is a part of innate immunity initiated by the immune cells that persists only for a short time. However, if the inflammation continues, the second stage of inflammation called chronic inflammation commences which instigates various kinds of chronic diseases, including arthritis, cancer, cardiovascular diseases, diabetes, and neurological diseases via dysregulation of various signaling pathways. Therefore reducing inflammation by therapeutic strategies would decrease the risk of various chronic diseases. Alpha-lipoic acid (ALA) is a natural antioxidant compound which is naturally found in plant and animal sources but small quantity of ALA can be absorbed as free ALA. The pivotal action of ALA is the antioxidant activity due to its ability to scavenge and inactivate free radicals, protecting against oxidative damage in several diseases, including neurodegenerative disorders.

New evidence suggests that ALA might be a useful supplement for inflammation induced by oxidative stress in chronic diseases. This study investigated whether ALA has a protective role under oxidative stress induced inflammation in *Danio rerio*. Zebrafish has emerged as a powerful model system to examine mechanisms of human disease. The presence of both innate and adaptive utility in zebrafish allows its use as a tool to examine the role of immune cells in normal development and in the pathogenesis of disease states. A gene expression analysis of several proinflammatory marker genes (il4, il13, tnfa, ifng1, nos2b) was carried out in adult zebrafish gut after LPS (alone) and LPS plus ALA administration. Our preliminary data showed that ALA administration was capable to reduce ($p<0.001$) the inflammation induced by LPS treatment. Furthermore, we will also evaluate the effect of ALA on immunological aspects of chronic inflammation, using spint1a mutant zebrafish larval model which exhibit chronic skin inflammation characterized by epidermal hyperproliferation and neutrophil infiltration.

keywords: Chronic disease, inflammation, alpha Lipoic Acid, zebrafish, spint1 mutant

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P-004.

Immune response assessment of Atlantic salmon against *P. salmonis* in sea-cage farming centers

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Abstract
The monitoring of sea farming centers at the south of Chile was performed to obtain data on the expression of 39 immune-related genes related of Salmo salar in three fish organs: gill, spleen and head kidney. The data was obtained from farms with and without occurrences of outbreaks of *Piscirickettsia salm*onis, and a generalized mixed linear model (GMLM) was established, considering the environmental variables and fish necropsy. This model allowed (i) the establishment of a baseline of expression of immune response genes, and (ii) molecular gene markers of susceptibility to the pathogen, which is, if fish are P. salmonis positive. For the identification of the baseline expression of immune genes, the condition of normal (healthy) fish was defined and differences in the expression of these genes were established in the gill, spleen and head kidney. In addition, genes exhibiting temporal variation in their expression were identified and therefore, an annual historical reference of this value was considered to the baseline determination. Regarding the genes proposed as markers of P. salmonis infection, it was determined that the expression of the genes coding for TNF-α, cathelicidin, NLRX1 and IL-1β, in gills; cathelicidin and hepcidin in anterior kidney; and hepcidin and IL-10 in spleen are indicators of infected fish, and thus, susceptible to P. salmonis. This result also highlights the data obtained at the gill level, an easy-sampling organ in the field with validated molecular indicators. While these studies were based on the genes expression levels, we also obtained result on the availability at the protein level of several of these molecules, using specific antibodies obtained in this project. The GMLM will also allow to propose molecules whose increase in expression over time can be predictive indicators of P. salmonis infection. One of the molecular markers with the best application perspective and whose availability was also evaluated at the protein level is cathelicidin expressed in gills. The use of the proposed molecular tools in sea farming centers to evaluate the expression of gene markers will be useful
for the identification of critical windows for therapeutic treatment. Consequently, if the expression of gene markers is detected between reference values, it will indicate infection by *P. salmonis* with an associated probability, and industry could applied productive strategies such as the use of medicated diets.
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keywords: Immune response, P. salmonis, cathelicidin, gill immunity, culture center

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P-005.

Transcriptome profiling of Atlantic salmon kidney cells (ASK) after stimulation with poly (I:C) and infection with infectious Salmon Anemia Virus (ISAV)

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Abstract
Viral diseases are of great concern in fish farming. Close to 20% of salmon put out to sea are lost during production and a large part of this is due to infections. Oil-adjuvanted multivalent vaccines against bacteria/viruses are available and confer good protection against bacterial disease but efficiency against viral disease under field conditions has been questioned. Our goal is to test if known agonists for human Toll-like receptors (TLRs) can be used as adjuvants in vaccines formulations, against fish viruses. Poly (I:C) is a synthetic analog of double-stranded RNA (dsRNA) that mimics a viral infection and could be used for immunostimulation in therapeutics and vaccines. But before developing a vaccine it is necessary to understand more about how the immune system responds to these ligands.
Using ASK cells as an in vitro model, we have compared the transcriptome response of cells infected with ISAV and cells stimulated by poly (I:C) in different time points (12 and 48h). RNA sequencing analysis revealed a total of 3111 differential expressed genes (DEGs) in the treated groups compared to control. From these DEGs, 2815 and 1309 genes were differentially expressed in the poly (I:C) and, ISAV groups respectively. Poly (I:C) treated cells showed stronger response both at 12h and 48 hours when compared with ISAV infected cells. Using the recently annotated salmon genome, pathway and gene ontology (GO) enrichment analyses were performed using Ingenuity and the R package “ClusterProfiler”. Most of the shared DEGs were immune-related and were overrepresented in pathways and GO terms related with immune response and response against virus. Some genes were only differentially expressed in one of the groups (e.g., CD28 − poly (I:C) group and interleukin 1β − ISAV group) while others were related only with early or late response against virus or poly I:C, for example interferon (IFNa3) was only detected in early poly (I:C) group (12h) and late ISAV group (48h) . Our results can help to comprehend the molecular mechanism of Atlantic salmon immune response against ISA virus infection. They can also help identify biomarkers for ISA virus early detection and to investigate the possible role of poly (I:C) as adjuvant for future vaccines in aquaculture.
keywords: Atlantic salmon, RNA sequencing, Poly (I:C), Toll-like receptor, ISA virus

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