Abstract

Diseases are the main cause of economic losses in the aquaculture sector that's why researching and improving this immunomodulation technique is fundamental. In this research work, the immunomodulatory effect of the lipopolysaccharides (LPS) of *Vibrio alginolyticus* was evaluated in a species of commercial interest, the seabass, through the cytokines, one kind of humoral components. Three groups of fish were evaluated, one unstimulated control, another positive stimulated with Poly I: C and another stimulated with LPS. The results showed statistically significant differences between the stimulants, where it was observed that the expression of the inflammatory (IL-1β, IL-6 and TNFα) and anti-inflammatory (IL-10) cytokines was directly related. The antiviral response (Mx) was different between the treatments and it was seen that with LPS the stimulating effect was more prolonged in time, although weaker.

keywords: Immunomodulation, cytokines, seabass, immunostimulants and lipopolysaccharides

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Corresponding author.
E-mail address: felix.acosta@ulpgc.es (F. Acosta).

* This author has contributed equally to this work.

P-071.

Circadian rhythmic expression of TNF-α gene regulated by clock gene in the Japanese medaka (*Oryzias latipes*)

T. Onoue [1], *G. Nishi* [2], *J. Hikima* [2], *M. Sakai* [2], *T. Kono* [2,#].

[1] Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, 1-1 Gakuen kibanadai-nishi, Miyazaki 889-2192, Japan
[2] Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen kibanadai-nishi, Miyazaki 889-2192, Japan

Abstract

To date, little information is available on the effects of circadian oscillation on immune regulation in lower vertebrates, such as teleost fish. In the present study, regulation of circadian rhythmic expression of inflammatory cytokine: TNF-α gene by clock genes (Bmal1 and Clock1) was investigated using Japanese medaka (*Oryzias latipes*). Firstly, structural analysis of clock gene was performed, which revealed that medaka Bmal1 and Clock1 conserve functionally important domains, such as basic helix-loop-helix (bHLH) and period-aryl hydrocarbon receptor nuclear translocator-single-minded (PAS), seen in their counterparts in other vertebrates. Expression of medaka Bmal1, Clock1 and Per1 genes was confirmed in central and peripheral tissues. Moreover, the expression of these clock genes and TNF-α genes in medaka acclimated to a 12:12 light (L) - dark (D) cycle showed circadian rhythm. In addition, higher expression of TNF-α gene was detected in medaka embryo cells (Ol-Hdr R-e3) overexpressing Bmal1 and Clock1 genes. It was suggested that this increase was mediated by transcriptional regulation by clock proteins, which target E-box sequence in the cis-element of TNF-α gene as was detected by luciferase reporter gene assay. Moreover, in vitro head kidney stimulation with LPS at different zeitgeber time (ZT) under LD12:12 condition affected the degree of TNF-α gene expression, which shows high and low responsiveness to LPS stimulation at ZT18 and ZT10, respectively. These results suggested that medaka TNF-α exhibited circadian rhythmic expression regulated by clock proteins and its responsiveness against immune-stimulation depends on time zone.

keywords: Medaka, TNF-α, Circadian rhythm, Clock gene, Transcriptional regulation

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Corresponding author.
E-mail address: tkono@cc.miyazaki-u.ac.jp (T. Kono).

P-072.

Molecular characterizations and ligand-induced responds of type I interferon receptor (IFNR1) in orange-spotted grouper (*Epinephelus coioides*)

Z.-Z. Tang [1,2], *T.-Y. Wang* [1,2], *T.-Y. Chen* [1,2,3,#].

[1] Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan, Taiwan
[2] Translational Center for Marine Biotechnology, National Cheng Kung University, Tainan, Taiwan
[3] Agriculture Biotechnology Research Center, National Cheng Kung University, Tainan, Taiwan

Abstract

Grouper fishes are known as high economical species in aquaculture industry. However, outbreak of diseases caused chronic death rate of grouper juveniles. To increase survival rate of grouper, mechanism of first line of defense against virus diseases in teleost fish is studied. Previous result indicated up-regulation of gene expression level of grouper IFN and downstream antiviral Mx protein gene in response to NNV virus infection. However, signalling cascade of IFN system is unclear. To investigate relationship between type I 2C-IFN to its receptor (IFNR), full length orange-spotted grouper IFNR1 was firstly cloned and identified. Phylogenetic analysis indicated osgIFNR1 shared high homology similarities with other teleosts. Relative expression of osgIFNR1 was determined using real-time qPCR in regards to interferon treatment using immune organ primary cell culture. Gene expression of osgIFNR1 and downstream osgMx1 which act as marker was seen up-regulated approximately 0.5 and 10-fold respectively in 4 hours post-stimulation indicating involvement of transcriptional level responses. We also observed transcript level of osgIFNR1 in response to immune-stimulant (LPS and Poly I:C) with increased fold of around 2 and 6 times respectively and nodavirus infected larvae of around 0.5-fold downregulation. We further used GF-1 cell line to observe subcellular localization and interaction between ligand and receptor. The present study revealed effect of IFNR1 through up-regulation of gene expression level during 4 hours of interferón (IFN) treatment. This study contributed different insights in analysis of IFNR role in type I interferón system in orange-spotted grouper.

keywords: Orange-spotted grouper, interferon receptor, nervous necrosis virus, immune system, molecular cloning

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Corresponding author.
E-mail address: ibcty@mail.ncku.edu.tw (T.-Y. Chen).

P-073.

Effects of recombinant IL-4/13A on the phagocytic capacity of salmonid leukocytes

V. Wong [#], *K. Maisey, R. Montero, M. Imarai* [#].

Laboratorio de Inmunología, Centro de Biotecnología Acuícola, Universidad de Santiago de Chile, Santiago, Chile

Abstract

The professional phagocytes described on teleost fish are macrophages, neutrophils and dendritic cells, and B lymphocytes. In mammals, several cytokines have effects on the phagocytic capacity of these cells, for example, IL-4 induces increase of phagocytosis by macrophages, as well as increase of reactive oxygen species production. In fish, it has been reported that IL-4/13 increases phagocytic capacity in Japanese pufferfish and Grass carp, however the function of this cytokine in salmonids has not been studied. In this work we evaluated the effect of recombinant IL-4/13A on the phagocytic capacity and ROS production of SHK-1 and

RTS-11 cell lines and salmon spleen B cells. The phagocytic capacity was evaluated with fluorescent latex beads and ROS production with the probe 2,7-dichlorohydrofluroescein diacetate by flow cytometry. The results showed that rIL-4/13A increases the percentage of phagocytosis in trout and salmon spleen B cells. Also this protein, increases the phagocytic capacity in RTS-11 and SHK-1 cell lines, and in addition the mean fluorescence intensity of all tested cells. Besides, rIL-4/13A increases ROS production in RTS-11 cells and trout and salmon spleen B cells. In conclusion, rIL-4/13A increases phagocytosis and the production of reactive oxygen species indicating that this cytokine plays a role stimulating phagocytes to effectively engulf and eliminate invading microorganisms in salmonids.

Corresponding authors.
E-mail addresses: valentina.wong@usach.cl (V. Wong), monica.imarai@usach.cl (M. Imarai).

P-074.

Atlantic salmon lymphocytes: Infection target cells for *Piscirickettsia salmonis*?

V. Wong[#,*], *M. Cortes*[*], *C. Navarro, M. Imarai*[#].

Laboratorio de Inmunología. Centro de Biotecnología Acuícola.
Universidad de Santiago de Chile, Santiago, Chile

Abstract

Piscirickettsia salmonis is a facultative intracellular gram-negative bacterium of variable size (0.5-1.5 μm of diameter) causing salmonid rickettsial septicaemia in salmonids. During 2017, *P. salmonis* was responsible of almost 70% of mortality of salmon due to infectious causes in Chilean marine aquaculture centers. The pathogen produces prominent lesions in the liver, kidney, spleen and intestine, and one of the main target cells are macrophages. To date, only few studies investigate the cell targets of infection and the strategies and kinetics of *P. salmonis* infection at the cellular level. Thus, this study aimed to characterize *P. salmonis* infection of Atlantic salmon leukocytes using confocal microscopy and flow cytometry. We hypothesized that not only macrophages, but also lymphocytes were able to internalize *P. salmonis*. Thus, leukocytes isolated from the spleen of Atlantic salmon were infected with *P. salmonis*-727 conjugated to FITC (MOI 10). First, a time-course study was performed to follow up salmon splenocytes infection with *P. salmonis* for 6 hours using confocal microscopy. Video recording showed that 5 min post-infection (p.i.), *P. salmonis* was attached to the cell membrane of salmon splenocytes, whilst the bacteria were on the cell surface and/or internalized 30 min p.i. Thirty minutes later almost all splenocytes had the bacteria internalized. During the 6 hours of infection, splenocytes of different size showed protrusions of cell membrane that seems to engulf the bacteria, which is a characteristic process during phagocytosis of a pathogen. Apoptotic bodies and dead cells were also observed during this period of time. We then examined if the lymphoid population internalize the bacterium. This was evaluated by flow cytometry, using salmon spleen lymphocytes, infected with *P. salmonis* 727-FITC (MOI 10) for 0.5, 4, 8 and 24 hours. The percentage of lymphocytes able to incorporate *P. salmonis*-FITC was evaluated. An increase in the number of live lymphocytes containing the bacteria was observed during the first 8 h, that was decreasing when approaching 24 hours after infection. Similarly, an increase in the percentage of dead lymphocytes with *P. salmonis* was observed, supporting results observed in confocal microscopy. In summary, we reported that spleen lymphocytes of Atlantic salmon can internalize *P. salmonis* that might be due to the phagocytic capabilities of fish lymphoid cells or because they are target cells for *P. salmonis* infection or both.

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keywords: Atlantic salmon, lymphocytes, *Piscirickettsia salmonis*, infectivity, phagocytosis.

Corresponding authors.
E-mail addresses: valentina.wong@usach.cl (V. Wong), monica.imarai@usach.cl (M. Imarai).
* These authors have contributed equally to this work.

P-075.

Comparative modulation of lncRNAs in wild-type and RAG1 heterozygous mutant zebrafish exposed to an immune challenge with Spring Viremia of Carp Virus (SVCV)

V. Valenzuela-Muñoz[1,*], *P. Pereiro*[1,2,*], *M. Álvarez-Rodríguez*[2], *C. Gallardo-Escárate*[1], *A. Figueras*[2], *B. Novoa*[2,#].

[1] Laboratory of Biotechnology and Aquatic Genomics, Interdisciplinary Center for Aquaculture Research (INCAR), University of Concepción, Concepción, Chile
[2] Instituto de Investigaciones Marinas (IIM), CSIC, Vigo, Spain

Abstract

Although the modulation of immune-related genes after viral infection have been largely described in vertebrates, the potential implication noncoding RNAs (ncRNAs), and especially the long non-coding RNAs (lncRNAs), in the immunity is still an incipient research field. The model species zebrafish could serve as a very useful organism to study the functionality of lncRNAs due to the numerous advantages of this teleost, including the existence of numerous mutant lines. In this work, we conducted whole-transcriptome analysis of kidney from wild-type (WT) and heterozygous *rag1* mutants (*rag1*+/-) after infection with the pathogen Spring Viremia of Carp virus (SVCV). The RAG1 of vertebrates is one of the endonucleases with a role in the assembly of immunoglobulins and T cell receptor (TCR) genes. The absence of functional RAG1 protein results in the impossibility to develop mature B and T lymphocytes. However, heterozygous *rag1* zebrafish, which are partially deficient in Rag1, could allow us to detect lncRNAs potentially involved in the adaptive immunity due to the compensatory processes induced after infection. We identified 12,165 putative lncRNA in zebrafish, most of them shared by both zebrafish lines. However, by comparing the lncRNA profile induced after SVCV infection in both WT and *rag1*+/-, we found that the majority of the lncRNAs significantly induced after viral challenge were exclusive of each line, reflecting a highly differential response to the virus. Analysis of the neighbor genes of lncRNAs exclusively modulated in WT revealed a high representation of metabolism-related terms, whereas those from *rag1*+/- showed enrichment in terms related to the adaptive immune response, among others. As was expected, commonly modulated lncRNAs were surrounded by genes involved in numerous antiviral processes. These results clearly indicate that, after SVCV infection, zebrafish are able to induce the expression of an array of lncRNAs with a function in different aspects of the immunity.

keywords: zebrafish, lncRNAs, RNA-Seq, SVCV, immunity, rag1

Corresponding author.
E-mail address: beatriznovoa@iim.csic.es (B. Novoa).
* These authors have contributed equally to this work.

P-076.

Two regulator of complement activation group 2 genes in rainbow trout: Gene duplication and divergent evolution

Peng Wang[1,2,*], *Pei Wang*[1,*], *Si-Zhe Fu*[2,*], *Xiang-Yang Zhang*[2], *Jun Li*[3,4], *Yong-An Zhang*[1,2,4,5,#], *Xu-Jie Zhang*[1,2,4,5,#].

[1] Department of Aquatic Animal Medicine, College of Fisheries, Huazhong Agricultural University, Wuhan, China
[2] Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China