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Temperature modulates the immunological response of the sub-antarctic notothenioid fish *Eleginops maclovinus* injected with *Piscirickettsia salmonis*

D. Martínez[a,b,c,**], C. Vargas-Lagos[a,c,d], R. Oyarzún[a,b,c], C.A. Loncoman[g,h], J.P. Pontigo[a], A.J. Yáñez[e,f], L. Vargas-Chacoff[a,c,*]

[a] Instituto de Ciencias Marinas y Limnológicas, Laboratorio de Fisiología de Peces, Universidad Austral de Chile, Valdivia, Chile
[b] Escuela de Graduados, Programa de Doctorado en Ciencias de l6a Acuicultura, Universidad Austral de Chile, Av. Los Pinos s/n Balneario Pelluco, Puerto Montt, Chile
[c] Centro Fondap de Investigación de Altas Latitudes (IDEAL), Universidad Austral de C'hile, Casilla 567, Valdivia, Chile
[d] Escuela de Graduados, Programa de Magister en Microbiología, Universidad Austral de Chile, Valdivia, Chile
[e] Centro Fondap Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Austral de Chile, Valdivia, Chile
[f] Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Valdivia, Chile
[g] Asia Pacific Centre for Animal Health, Faculty of Veterinary Science, University of Melbourne, Melbourne, Australia
[h] Applied Biochemistry Laboratory, Institute of Pharmacology and Morphophysiology, Faculty of Veterinary Sciences, Universidad Austral de Chile, Valdivia, Chile

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ABSTRACT

Eleginops maclovinus is a eurythermic fish that under natural conditions lives in environments with temperatures ranging from 4 to 18 °C and can be usually captured near salmon farming areas. The aim of this study was to evaluate the effect of temperature over the innate and adaptive immune response of *E. maclovinus* challenged with *Piscirickettsia salmonis* following different treatments: C− (control injection with culture medium at 12 °C), C+ (bacterial injection at 12 °C), 18 °C c/A + B (injection with culture medium in acclimation at 18 °C), 18 °C c/A + B (bacterial injection in acclimation at 18 °C), 18 °C s/A + M (injection with culture medium without acclimation at 18 °C) and 18 °C s/A + B (bacterial injection without acclimation at 18 °C). Each injection had 100 μL of culture medium or with 100 μL at a concentration 1×10^8 of live bacteria, sampling six fish per group at 4, 8, 12, 16 and 20 days post-injection (dpi). Expression of the mRNA related with the innate immune response gene (TLR1, TLR5, TLR8, NLRC3, NLRC5, MyD88 and IL-1β) as well as the adaptive immune response gene (MHCI, MHCII, IgMs and IgD) were measured in spleen and head kidney. Gene expression profiles were treatment-type and time dependent. Levels of Immunoglobulin M (IgM) increased in challenged groups with *P. salmonis* from day 8–20 post challenge, which suggest activation of B cells IgM + through *P. salmonis* epitope detection. Additionally, a rise in temperature from 12 °C (C+) to 18 °C (with/without acclimation) also resulted in antibody increment detected in serum with significant differences between "18 °C c/A + B" and "18 °C s/A + B" groups. This is the first study that evaluates the effect of temperature changes and mRNA expression related with immune system gene over time on *E. maclovinus,* a native wild life fish that cohabits in the salmon farming environment.

1. Introduction

The sudden or extreme changes in the environmental temperature can exert a species-specific effect on the immune system of the ectotherm fish [1,2]. As in mammals, the immune system is divided into innate or nonspecific and adaptative or specific, both composed by humoral and cellular components [3] that involve primary (head kidney) and secondary (spleen-head kidney-MALT) lymphoid organs [4]. The cells and molecules of the innate immune system use non-clonal pattern recognition receptors, including C-type lectin receptors, Toll-like receptors (TLRs), and NOD-like receptors (NLRs) [5]. Compared to the cells of the adaptive immune system, which expresses clonal receptors, these are specifically able to recognize antigens and their derived peptides [5]. Both systems communicate through soluble mediators known as cytokines, which mediate the immune response and alert the recognition of a pathogenic stressor, rapidly controlling pathogen growth, promoting inflammation, and triggering an adaptive immune response [5].

* Corresponding author. Instituto de Ciencias Marinas y Limnológicas, Laboratorio de Fisiología de Peces, Universidad Austral de Chile, Valdivia, Chile.
** Corresponding author. Instituto de Ciencias Marinas y Limnológicas, Laboratorio de Fisiología de Peces, Universidad Austral de Chile, Valdivia, Chile.
E-mail addresses: danixapamela@gmail.com (D. Martínez), luis.vargas@uach.cl (L. Vargas-Chacoff).

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The fish utilize preferably their innate immune response in circumstances of low environmental temperature whereas at high temperatures they use their adaptive immune system [1,6–10]. The immune response most affected by temperature is the activation of T lymphocytes and the production of antibodies dependent, although the components of innate immunity can also respond by decreasing the lytic activity of complement and serum levels of lysozyme and protein C reactive [11]. This antecedents shows that the immunological response of ectothermic fish must function in a wide range of environmental temperatures, in order to adequately respond to infectious diseases that occur in thermal situations where the replication of the pathogen is favored [12].

Piscirickettsia salmonis is the etiological agent of Piscirickettsiosis, a disease that causes high mortalities in the aquaculture industry [13]. This bacterium facultative intracellular [14–18] generates a systemic infection characterized by colonization in several organs, including the kidney, liver, spleen, intestine, ovary, gills, and brain [13]. *P. salmonis* replicates within cytoplasmic inclusions in host cells by binary fission, causing a cytopathic effect (CPE) on salmon cell lines, with an *in vitro* replication that is optimal at 15 °C and 18 °C, slower at 21 °C and 10 °C and stops at 4 °C [19]. Piscirickettsiosis was initially reported as a disease in salmonids only, however, evidence exists in other non-salmonid species, such as *Dicentrarchus labrax* [20], *Atractoscion nobilis* [21], *Oreochromis mossambicus* and *Sarotherodon melanotheron* [22]. Genetic material of this bacterium has even been detected in fish native to Chile, including *Eleginops maclovinus*, *Odontesthes regia*, *Sebastes capensis* and *Salilota australis* [23]. The role that these native fish could play in disease transmission, as well as the effects that this bacterium could have on the tissues of these endemic organisms, is unknown.

In salmonids, *P. salmonis* can infect, survive, replicate, and disseminate within host monocytes/macrophages without inducing a cytopathic effect [24], causing macrophage apoptosis during the early, intermediate and late stage of the infection cycle [25]. The transcriptional responses of macrophages infected *in vitro* with *P. salmonis* and of the head kidney of Atlantic salmon, 2 days [26] and 14 days [27] after intraperitoneal (i.p.) infection with *P. salmonis* have been investigated using microarrays. These investigations revealed an overexpress of genes involved in the inflammatory and oxidative responses and underexpresses of genes implicated in the adaptive immune response, and apoptotic processes [26,27]. Additionally, in *E. maclovinus* has been described that the administration of total proteins from *P. salmonis* cause metabolic, osmorregulatory and immunological modifications [28]. Furthermore, the administration of live *P. salmonis* by the intraperitoneal route in this species results in modulation of iron metabolism [29,30] and an induction of innate and adaptative immune response [31].

Up to date, the effect of sudden (shock) or gradual (acclimation) temperature variation in the environment over the immune response against *P. salmonis* remains unknown, this is pivotal as temperature may impact on mortality rates in group of fish infected with *P. salmonis* [32]. Fort et al. [33] reported that *E. maclovinus* is usually captured near salmon farming areas, where apparently they obtain food out of nutrient wastes produced by the salmon farming activity. This proximity with salmon farms areas suggest an environmental interaction between *E. maclovinus* and farmed salmon fishes, which may result in a risk of transmission of pathogens such as *P. salmonis* [23].

E. maclovinus (Valenciennes 1930) is a sub-Antarctic notothenioid of the family Eleginopsidae suborder Notothenioidei that is capable of supporting a wide thermal range in its natural environment, therefore it is considered eurythermic [34]. Several physiological processes of this species are influenced by environmental temperature [35–37]. In this study, we aimed to evaluate the innate and adaptive immune response over time in lymphoid tissue organs after different temperature challenges in *E. maclovinus* infected with *P. salmonis*.

2. Materials and methods

2.1. Samples

E. maclovinus (20 ± 5 g body weight) individuals were transferred to the Laboratory of Aquatic Pathology and Biotechnology, Faculty of Veterinary Sciences, Animal Pathology Institute, Universidad Austral de Chile. Fish were acclimated (four weeks) in 500 L tanks with seawater (32 psμ, 1085 mOsm kg^{-1}) and at a density of 3.1 kg m^{-3}, following indications given by Vargas-Chacoff et al. [38]. Fish were fed once daily with a 1% body weight ratio of commercial dry pellets (Skretting Nutrece 100 ML; containing 48% protein, 22% fat, 13.5% carbohydrates, 8% moisture, and 8.5% ash).

The experiments described herein were performed following the standards of the Guide for the Care and Use of Laboratory Animals of the National Commission of Science and Technology (CONICYT, Chile) and the Universidad Austral de Chile. The Ethics protocol was approved by the Committee on the Ethics for Animal Experimentation of Universidad Austral de Chile Memorandum N° 261/2016.

2.2. Primers design

All steps involving massive cDNA sequencing were published by Martínez et al. [39]. In detail, total RNA was extracted from one tissue (head kidney of a healthy fish) with the commercial RNA NucleoSpin® RNA Kit (Macherey-Nagel). RNA was selected with optimal integrity (RIN > 7). Subsequently, mRNA isolation was performed with the PolyATtract III Kit® in an mRNA Isolation System (Promega). The quantity and quality of total mRNA was evaluated using an A260 ND-1000 spectrophotometer (NanoDropH Technologies) and Agilent 2100 Bioanalyzer (Agilent Technologies), respectively. The cDNA library was constructed following the "Rapid cDNA library preparation" protocol recommended by Roche. Bioinformatics analysis of the obtained data included the formation of transcripts (contigs); specifically by using a combination of specialized transcriptomic analysis software followed by transcript annotation against the non-redundant NCBI database. After this process, mRNA sequences of MHCI, MCHII, NLRC3, NLRC5, TLR1, TLR8, IgMs, and IgD were obtained and analyzed using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/). RNA-seq revealed some genes implicated in the immune response, nevertheless, the other genes selected for study were amplified from heterologous primers (IL-1β, TLR5, and MyD88). Genes related to the innate immune response included TLR (1, 5, and 8), NLRC (3 and 5), MyD88 and IL-1β. Genes related to the adaptive immune response were MHCI, MHCII, IgMs and IgD. Partial cDNA coding sequences were obtained and deposited in GenBank with the accession numbers indicated in Table 1.

2.3. Psalmonis LF-89

P. salmonis LF-89 strain (ATCC® VR-1361™) was kindly donated by the Laboratory of Metabolism and Biotechnology, Institute of Biochemistry and Microbiology, Faculty of Sciences, Universidad Austral de Chile (Valdivia, Chile). This bacterium was grown in AUSTRAL-TSFe agar plates and incubated at 18 °C for ten days, according to standard conditions [17]. Subsequently, the colonies were grown in 4.5 mL AUSTRAL-SRS broth at 18 °C for 72 h with moderate agitation (75 rpm) [18].

2.4. Infection assays with P. salmonis LF-89

After acclimation the fish were randomly distributed among rectangular tanks (100 L) for subsequent application of the following experimental treatments in duplicate (n = 180 total): C− at 12 °C (control injection with culture medium at 12 °C), C+ at 12 °C (bacterial injection at 12 °C), 18 °C c/A + B (injection with culture medium in acclimation at 18 °C), 18 °C c/A + B (bacterial injection in acclimation at