

IgM) and IgM-IgD-IgT+ cells. The IgM+ and IgT+ cells were scattered throughout the parenchyma, and grouped around large vessels and surrounding melanomacrophage centres (MMC). Cell proliferation was estimated using a combination of anti-IgT, anti-IgM and anti-PCNA antibodies. Proliferation of both IgM+ and IgT+ B cells was observed in several areas of the spleen. In addition, vaccinated fish showed a mass of vaccine and cells (CVM) associated to the spleen. The CVM contained scattered PCNA+/IgM+ and PCNA+/IgT+ B cells, possibly indicating its importance during vaccination, above and beyond its role as a site for phagocytosis and material exchange.

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keywords: Turbot, Spleen, vaccination, B lymphocytes, Immunoglobulins

Corresponding author.

E-mail address: jesus.lamas@usc.es (J. Lamas).

P-043.

Protective immune responses of recombinant outer membrane proteins OmpF and OmpK of *Aeromonas hydrophila* in European eel (*Anguilla anguilla*)

W. Zhang^{1,2}, Z. Liao¹, F. Hu², X. Chen^{2,3,#}, X. Huang^{*1,#}.

¹ University Key Lab for Integrated Chinese Traditional and Western Veterinary Medicine and Animal Healthcare in Fujian Province, Fujian Agriculture and Forestry University, Fuzhou 350002, China

² Institute of Oceanology, Fujian Agriculture and Forestry University, Fuzhou 350002, China

³ Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, China

Abstract

Outer membrane proteins (Omps) of Gram-negative bacteria were proved to be efficient subunit vaccines against bacteriosis. In this study, OmpF and OmpK of *Aeromonas hydrophila* were expressed and evaluated their immune protective effects on European eel (*Anguilla anguilla*). The genomic DNA of *A. hydrophila* 322A was used as the template, and two kinds of prokaryotic expression plasmids pET-32a-OmpF and pET-32a-OmpK were constructed, respectively. Recombinant OmpF protein (r-OmpF) and r-OmpK were purified and proved to have antigenicity by Western-blot analysis. The r-OmpF and r-OmpK were used as immunogens to immunize European eel by the intraperitoneal injection. The mRNA expression of 6 immune-related genes (*IgM*, *IL-10*, *IRF3*, *IRF7*, *LysG4*, and *HexB*) in liver tissues of eels at 1 h, 3 h, 6 h, 12 h, 24 h, 72 h, and 10 d post-immunization was analyzed by real-time PCR. At 30 dpi, serum antibody response was measured by ELISA. Fish were attacked at 15 dpi by live 322A in order to assess the protective immunity of r-OmpF and r-OmpK. Both r-OmpF and r-OmpK could up-regulate the expression of all 6 genes in varying degrees. The serum antibody titer of r-OmpF- and r-OmpK-immunized groups was 1: 1600 and 1: 3200, respectively. In addition, r-OmpF could give 35.5% of relative immune protection rate to European eels, while r-OmpK gave 70.0%. By analyzing the protective immunity and the regulatory role in the immune-related gene expression of the two recombinant proteins provided, it could be found that r-OmpK was a potential vaccine candidate of *A. hydrophila*.

keywords: *Aeromonas hydrophila*; outer membrane protein; subunit vaccine; protective immunity; European eel (*Anguilla anguilla*)

Corresponding author.

E-mail addresses: chenxinhua@tio.org.cn (X. Chen), xhhuang138@hotmail.com (X. Huang).

P-044.

Transcriptome analysis of immune-related gene expression in hybrid snakehead (*Channa maculata* ♀ X *Channa argus* ♂) after challenge with *Nocardia seriolae*

J.L. Chen^{1,2,3,4}, Y.Q. Li², W.J. Wang^{1,2,3,4}, L.Q. Xia^{*1,2,4,#}, Z.W. Wang^{1,2,3,4}, S.Y. Hou^{1,2,3,4}, J.H. Huang², Y.S. Lu^{*1,2,3,4,#}.

¹ Shenzhen Research Institute of Guangdong Ocean University, Shenzhen, China

² Fisheries College of Guangdong Ocean University, Zhanjiang, China

³ Guangdong Provincial Key Laboratory of Pathogenic Biology and Epidemiology for Aquatic Economic Animals, Zhanjiang, China

⁴ Guangdong Provincial Engineering Research Center for Aquatic Animal Health Assessment, Shenzhen, China

Abstract

Hybrid snakehead fish (*Channa maculata* ♀ x *Channa argus* ♂), a new species used in freshwater aquaculture in China, is the common host of an epizootic bacterial infection by *Nocardia seriolae*. However, the information on the functions and mechanisms of hybrid snakehead immune pathways with the *N. seriolae* infection is limited. Thus, the peripheral blood lymphocytes from hybrid snakehead were used for transcriptome analysis to understand the host immune response after challenge with *N. seriolae*. A total of 49,839,332 and 50,059,283 raw reads were obtained from the *N. seriolae*-challenged group (Ns group) and phosphate-buffered saline control group (Ctr group), respectively. The 75.50% and 74.25% reads from the Ns and Ctr groups were matched to reference genomic sequence after cleaning the raw reads, respectively. Additionally, there were 2892 significant differentially expressed genes (DEGs) among the 17,196 expressed genes between the Ns and Ctr groups, including 1387 upregulated and 1505 downregulated genes. All the DEGs were classified into three gene ontology categories, and 2502 DEGs had significant matches, which were allocated to 246 Kyoto Encyclopedia of Genes and Genomes pathways. Immune-related genes were detected from immune system pathways among the top 20 enriched pathways. Moreover, the regulation of several observed effective genes was confirmed by real-time quantitative Polymerase chain reaction. Altogether, this study offers deep-sequence data of hybrid snakehead peripheral blood lymphocyte via transcriptome analysis and lays the foundation for further study on the immunogenetics of hybrid snakehead. Moreover, it provides insights into the pathogenic mechanism of *N. seriolae*, facilitating the prevention and treatment of fish nocardiosis.

keywords: *Nocardia seriolae*, Hybrid snakehead, Transcriptome analysis, Immune-related genes, Fish nocardiosis

Corresponding author.

E-mail addresses: xialq@gdou.edu.cn (L.Q. Xia), 11465668@qq.com (Y.S. Lu).

P-045.

A recombinant vaccine targeting the parasitic ciliate *Ichthyophthirius multifiliis*

L.V.G. Jørgensen^{1,#}, P.W. Kania¹, D.A.S. Araneda², N. Lorenzen², A. Stratmann³, K. Buchmann¹.

¹ Laboratory of Aquatic Pathobiology at the Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark

² DTU AQUA, National Institute of Aquatic Resources, the Technical University of Denmark

³ W42 Industrial Biotechnology GmbH, Dortmund, Germany

Abstract

New vaccine candidates were identified targeting the one celled parasite *I. multifiliis*, which negatively affects aquaculture freshwater fish productions all over the world. In silico selection with the use of artificial

intelligence identified several potential vaccine candidates and three of these were recombinantly expressed using *E. coli* and insect cells. Following a vaccine trial one protein (a so-called neurohypophysial n-terminal domain protein, #10) was found to induce moderate protection against *I. multifiliis* in rainbow trout (*Oncorhynchus mykiss*). To develop a highly protective heterologous vaccine we aim to combine #10 with a protective epitope from the already known homologous protective antigen lag52b, which is a GPI-anchored cysteine rich surface protein. To be able to produce #10 at low costs, recombinant expression has been conducted in an eukaryotic host. Purified lag52b does not induce immunity in fish without the use of adjuvants, thus the most potentially protective epitope of lag52 was selected in silico and coupled to a viruslike particle. This coupling enables the epitope to be presented in a virus-like conformation, which theoretically should be immunogenic to the fish. Results are discussed.

keywords: Recombinant vaccine, Ichthyophthirius multifiliis, protective epitope, vaccine candidates, virus-like particle

Corresponding author.

E-mail address: lvj@sund.ku.dk (L.V.G. Jørgensen).

P-046.

Transcriptomic profiles of post-smolt Atlantic salmon challenged with *Piscirickettsia salmonis* reveal a strategy to evade the adaptive immune response and modify cell-autonomous immunity

M. Rozas-Serri^{1,2,#}, A. Peña^{1,#}, L. Maldonado¹.

¹Laboratorio Pathovet Ltda., Puerto Montt, Chile

²Newenko Group SpA., Puerto Montt, Chile

Abstract

Piscirickettsiosis is the main bacterial disease affecting the Chilean salmon farming industry and is responsible for high economic losses. The development of effective strategies to control piscirickettsiosis has been limited in part by insufficient knowledge of the host response. The aim of this study was to use RNA sequencing to describe the transcriptional profiles of the responses of post-smolt Atlantic salmon infected with LF-89-like or EM-90-like *Piscirickettsia salmonis*. Enrichment and pathway analyses of the differentially expressed genes revealed several central signatures following infection, including positive regulation of DC-SIGN and TLR5 signalling, which converged at the NF- κ B level to modulate the pro-inflammatory cytokine response, particularly in the PS-EM-90-infected fish. *P. salmonis* induced an IFN-inducible response (e.g., IRF-1 and GBP-1) but inhibited the humoral and cell-mediated immune responses. *P. salmonis* induced significant cytoskeletal reorganization but decreased lysosomal protease activity and caused the degradation of proteins associated with cellular stress. Infection with these isolates also delayed protein transport, antigen processing, vesicle trafficking and autophagy. Both *P. salmonis* isolates promoted cell survival and proliferation and inhibited apoptosis. Both groups of Trojan fish used similar pathways to modulate the immune response at 5 dpi, but the transcriptomic profiles in the head kidneys of the cohabitant fish infected with PS-LF-89 and PS-MS-90 were relatively different at day 35 post-infection of the Trojan fish, probably due to the different degree of pathogenicity of each isolate. Our study showed the most important biological mechanisms used by *P. salmonis*, regardless of the isolate, to evade the immune response, maintain the viability of host cells and increase intracellular replication and persistence at the infection site. These results improve the understanding of the mechanisms by which interacts with its host and may serve as a basis for the development of effective strategies for the control of piscirickettsiosis.

keywords: RNA-seq, Piscirickettsiosis, *Piscirickettsia salmonis*, LF-89, EM-90.

Corresponding author

E-mail address: marco.rozas@pathovet.cl (M. Rozas-Serri).

P-047.

Pivotal role of immunoglobulin IgT in rainbow trout skin after bacterial infected with *Flavobacterium columnare*

X.T. Zhang, Z. Xu[#].

Department of Aquatic Animal Medicine, College of Fisheries, Huazhong Agricultural University, Wuhan, Hubei, 430070, China

Abstract

In contrast to mammalian skin, teleost skin has been considered as mucosal surface which serves as the first line of defense against invading pathogens. Moreover, teleost skin contains skin-associated lymphoid tissue (SALT) that elicits gut-like immune responses against parasitic infection. However, little is known so far about the B cells and immunoglobulins (Igs) responds to bacterial infection in the skin mucosal immune system of teleost. We hypothesized that, microbial exposure can elicit a dedicated mucosal Igs response and locally specific immune responses would be generated within its mucosa. To address our hypothesis, we construct an infected model with rainbow trout (*Oncorhynchus mykiss*), which was experimentally exposed to *Flavobacterium columnare*. H & E staining of trout skin shows the morphological changes and qRT-PCR indicates the increased mRNA expression levels of immune-related genes, which were further studied by RNA-Seq analysis, in trout skin after infected with *Flavobacterium columnare*. Moreover, strikingly increased IgT concentration and strong pathogen-specific IgT responses were detected in the cutaneous mucus, and the accumulation of IgT+ B cells were also noted in the skin epidermis of experimental group. Critically, IgT responses against the pathogen were mainly limited to the skin whereas IgM responses were almost exclusively detected in the serum. Moreover, local IgT+ B cells proliferation and pathogen-specific IgT generation were found in the trout skin, providing new evidence for the local mucosal immune responses in trout skin. Overall, our findings indicate that, following bacteria exposure, IgT and IgT+ B cells play the prevailing role in skin mucosal immunity. To our knowledge, our results provide the first example of locally induced immunoglobulin in the skin of rainbow trout after *Flavobacterium columnare* infection.

keywords: Skin, B cells, Immunoglobulins, *Flavobacterium columnare*, Rainbow trout (*Oncorhynchus mykiss*)

Corresponding author.

E-mail address: zhenxu@mail.hzau.edu.cn (Z. Xu).

P-048.

Early immune response in Atlantic salmon vaccinated with inactivated whole-cell bacterin of *Piscirickettsia salmonis* and pathogenic isolates

M. Rozas-Serri^{1,2,#}, A. Peña¹, L. Maldonado¹.

¹Laboratorio Pathovet Ltda., Puerto Montt, Chile

²Newenko Group SpA., Puerto Montt, Chile

Abstract

Piscirickettsiosis (SRS) is the main bacterial disease affecting the Chilean salmon farming industry. The aim of this study was to describe and comparatively quantify the immune response of Atlantic salmon intraperitoneally infected with LF-89 and EM-90 *Piscirickettsia salmonis* and vaccinated with inactivated whole-cell bacterin of *P. salmonis*. A positive correlation of the overexpression of IFN γ , IL-2, IL-10, IL-12 β , MHC-II and CD4 was seen in the PS-LF-89- and PS-EM-90-infected fish, but the proinflammatory response in the PS-EM-90-infected fish was more exacerbated. The fish infected with PS-LF-89 showed an anti-inflammatory response, whereas this finding was not observed in the PS-EM-90-infected fish. Conversely, a positive correlation of the downregulation of IFN γ , IL-2, IL-12 β , MHC-I and CD8 was seen in the vaccinated fish. Fish