

² Skretting Animal Research Centre, Stavanger, Norway

³ Aquaculture and Fisheries, Animal Sciences Group, Wageningen University and Research, Wageningen, Netherlands

Abstract

In the last decades, pollution of the environment by large scale use of antibiotics in agriculture and human medicine have led to increased antimicrobial resistance in both the environment and the host animal microbiome. Disturbances in the host microbiome can result in impaired immunity and reduced resilience of aquaculture species. Here, we investigated whether environmentally measured levels of the commonly used antibiotics ciprofloxacin and oxytetracycline influences the host microbiome and susceptibility toward saponin-induced immune stimulation in larval zebrafish. Firstly, neutrophil and macrophage reporter zebrafish larvae were exposed to different concentrations of soy saponin by immersion. A dose-dependent increase in neutrophil presence in the intestinal area was observed together with increased expression of immune genes *il1b*, *tnfa*, *il22* and *mmp9*. To investigate the effect of antibiotics, larval zebrafish were immersed in ciprofloxacin or oxytetracycline in the presence or absence of a low dose of saponin. In vivo imaging revealed that antibiotic treatment did not reduce the number of neutrophils that were recruited to the intestinal area upon saponin exposure, although it did tend to lower pro-inflammatory cytokine levels. Microbial sequencing of whole larvae revealed that exposure to a low dose of saponin already shifted the microbial composition. The combination of oxytetracycline and saponin significantly increased α -diversity compared to the controls. In conclusion, the current study provides evidence that the combination of low levels of antibiotics with low levels of anti-nutritional factors (saponin) can induce inflammatory phenotypes and can modify the microbiota, which might lead to altered disease susceptibility.

Keywords: Zebrafish, microbiota, saponin, neutrophils, macrophages, antibiotics

Corresponding author.

E-mail address: Sylvia.brugman@wur.nl (S. Brugman).

O-008.

Can passive immunization prevent disease outcome in gilthead sea bream exposed to *Enteromyxum leei*?

A. Picard-Sánchez, I. Estensoro, R. del Pozo, M.C. Piazzon[#], A. Sitjà-Bobadilla.

Fish Pathology Group, Instituto de Acuicultura de Torre de la Sal (IATS), Consejo Superior de Investigaciones Científicas (CSIC), Castellón, Spain

Abstract

Passive immunization is used in humans for treating or preventing some infectious diseases, but it also constitutes an emerging field of interest in aquaculture, particularly with the restrictions for antibiotic use. Intracoelomically-injected antibodies can be detected in fish sera within the first 8 h and their half-life ranges from 7 to 22 days post-injection, depending on the species. The fish models studied so far used fast-acting pathogens such as virus, bacteria or ciliate parasites. The current work aimed to determine if passive immunization could help to prevent enteromyxosis in gilthead sea bream (GSB, *Sparus aurata*). *Enteromyxum leei* is a myxozoan intestinal parasite that invades the paracellular space of the intestinal epithelium, producing a slow-progressing disease, leading to anorexia, cachexia and mortalities. We have previously demonstrated that GSB that survive *E. leei* infection become resistant upon re-exposure, and this resistance is directly related to the presence of high levels of specific serum antibodies.

In the current study, we evaluated whether injection with sera from resistant animals would protect naïve fish when challenged by effluent exposure to the parasite. Serum from a pool of resistant (R) and naïve (N) animals (intact or heat inactivated, 10 μ l/g BW) was intracoelomically injected 24 h prior to the *E. leei*-effluent challenge and at 9 days post-

challenge (dpc). At 23 dpc, the different groups were allocated in separate tanks and the effluent exposure was terminated. A non-lethal parasite diagnosis was performed at 56 dpc. At the final sampling (100 dpc), blood, serum and tissues were collected for hematology, circulating antibodies, histological and molecular diagnosis and gene expression.

Groups injected with R sera had lower prevalence and intensity of infection than those with N sera, both in the intermediate and final samplings. At 100 dpc, the prevalence of infection in the PBS and N groups was 70%, whereas in R group it only reached 55%. Condition factor (CF) and specific growth rate (SGR), key parameters affected by enteromyxosis, were higher in R group. There was a significant correlation between prevalence of infection and SGR and CF. Immunohistochemistry and gene expression studies will reveal whether this partial protection was due to higher presence of specific antibodies or specific cell populations. These results show that, even with this long term disease, passive immunization can confer some degree of protection. The administration of specific antibodies during exposure, probably provided fish with time to activate the specific defenses before the parasite proliferated.

ACKNOWLEDGEMENTS: This work was funded by EU H2020 program through ParaFishControl Project (634429) and Spanish MINECO project AGL2013-48560. M.C.P. was contracted under CSIC PIE project no. 201740E013 and FPD1-2013-15741 and I.E. under APOSTD/2016/037 grant by the "Generalitat Valenciana".

Keywords: Passive immunization, Myxozoa, antibodies, gilthead sea bream, parasites.

Corresponding author.

E-mail address: carla.piazzon@csic.es (M.C. Piazzon).

O-009.

Bacterial membrane vesicles as vaccines in aquaculture

J.I. Tandberg, L. Lagos, A. Kashulin, H. Sørum, H.C. Winther-Larsen[#].

^a Section for Microbiology, Immunology and Parasitology, Department of Food Safety and Infection Biology, Norwegian School of Veterinary Science, Oslo, Norway

^b Department of Biosciences, University of Oslo, Oslo, Norway

^c Laboratory for Microbial Dynamics and Center of Integrative Microbial Evolution, School of Pharmacy, University of Oslo, Oslo, Norway

^d Molecular Infection Medicine Sweden and Department of Clinical Microbiology, Umeå University, Umeå, Sweden

Abstract

Infections by two Gram-negative facultative intracellular bacterial pathogens, namely, *Piscirickettsia salmonis* and *Francisella noatunensis*, are causing major problems in aquaculture world-wide. *F. noatunensis* sp is one of the main factors hampering the development of fish farming based on Atlantic cod in Norway and is deleterious to tilapia, a farmed fish that is produced over 3.5 mill tons/year. *P. salmonis* infections have been devastating for salmon aquaculture. As of today no effective treatments are available against the diseases. The immunologically inaccessible intracellular location of *Francisella* and *Piscirickettsia* have until now complicated the development of protective measures. This is in stark contrast to the successful development of efficient vaccines that has been made possible against important extracellular bacterial infections in salmon based on whole inactivated bacteria injected with oil adjuvants. It has been shown that both *P. salmonis* and *F. noatunensis* secrete membrane vesicles (MV). Bacterial MVs has been shown to contain proteins, DNA and RNA and simulate the mother bacteria in a non-replicative form. Bacterial MV has been reported as potential vaccine candidates for a range of host including humans, mice and fish against infection caused by intracellular pathogenic bacteria as they induce both a humoral and cellular immunity. Here the characterization of MV isolated from *P. salmonis* and *F. noatunensis* is described, and their vaccine potential is verified in a zebrafish infection

and vaccine model. The further use of the MVs as vaccines in their natural hosts such as strain-specificity and cross-immunity will be discussed.

Corresponding author.

E-mail address: h.c.winther-larsen@farmasi.uio.no (H.C. Winther-Larsen).

O-010.

Potential role of rainbow trout red blood cells as mediators in the immune response induced by DNA vaccines

S. Puente-Marin¹, I. Nombela¹, V. Chico¹, S. Ciordia², M.C. Mena², J. Coll³, L. Mercado⁴, M. Ortega-Villaizán^{1, #}.

¹Instituto de Biología Molecular y Celular, Universidad Miguel Hernández (IBMC-UMH), Elche, Spain; Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche, Universidad Miguel Hernández, (IDiBE-UMH), Elche, Spain

²Unidad de Proteómica, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain

³Instituto Nacional de Investigaciones Agrarias (INIA), Biotecnología, Madrid, Spain

⁴Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

Abstract

Fish red blood cells (RBCs), unlike mammals, possess nucleus and organelles in their cytoplasm that give them the necessary machinery to generate an immune response at transcriptional and at proteomic level. In the last years nucleated RBCs have demonstrated to act as phagocytic cells, release cytokine-like factors and modulate leukocyte activity upon different stimulus. Also, they have been implicated in the response against viral infections. And recently, rainbow trout RBCs have been also implicated in the immune response to a DNA vaccine. So far, DNA vaccination is the best strategy to prevent and control viral infections, and for fish rhabdoviruses, only the DNA vaccine based on glycoprotein G (gpG) have resulted effective. However, the whole mechanisms involved in this protection and the immune response triggered by the DNA vaccine remain to be fully understood. In order to investigate the role of nucleated RBCs in DNA vaccination, we evaluated the immune response triggered by a DNA vaccine encoding the gpG of viral hemorrhagic septicaemia virus (VHSV) (GVHSV) in rainbow trout RBCs and explored RBCs as future targets or carriers for DNA vaccination. Upon fish vaccination of rainbow trout with GVHSV DNA vaccine, RBCs upregulated antigen presentation pathways at transcriptome and proteome level. In addition, rainbow trout RBCs responded to the DNA vaccine upregulating interferon type 1 (IFN-1) pathway. Also, RBCs transfected *in vitro* with GVHSV DNA vaccine protected RTG-2 cell line against subsequent viral infection. Besides, RBCs carrying the GVHSV DNA vaccine were able to induce specific antibody against VHSV *in vivo*. Also, RBCs transfected *in vitro* with GVHSV were able to modulate leukocyte activity *in vitro*. In summary, we suggest nucleated RBCs as cell mediators of the immune response playing an active role in DNA vaccination and propose nucleated RBCs as potential cell targets or carriers of antiviral prophylactics.

Keywords: Red blood cells, DNA vaccine, GVHSV, transcriptome, proteome

Corresponding author.

E-mail address: mortega-villaizan@umh.es (M. Ortega-Villaizán).

O-011.

Characterization of immune responses in different environmental temperature through linear array epitope vaccine induction

J.S.-W. Lam^{1, #}, T.-Y. Chen^{1, 2, 3}.

¹Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan, Taiwan

²Translational Center for Marine Biotechnology, National Cheng Kung University, Tainan, Taiwan

³Agriculture Biotechnology Research Center, National Cheng Kung University, Tainan, Taiwan

Abstract

Grouper fish have a very high value economically and also nutritionally; these make them a very important fish species across the world. However, this fish often suffers a very high mortality rate, up to 90%-100% whenever they are infected with a virus and always bring about a tremendous loss to the farmers of the relative fisheries. As fishes are ectothermic animals, where their bodily temperature and the immune system are regulated by the constant change of environmental temperature, we would like to characterize the vaccine in immune response induction upon virus infection in fish under various modulation of temperatures. Previously, our previous study has developed a vaccine NNVCP-S5E that was developed by a PCR based technique, linear array epitope (LAE) where the immunogen is having multiple linear epitope copies, which were predicted and chosen from NNV and the epitopes are amplified by template-repeat polymerase chain reaction (TR-PCR). Efficacy tests of the vaccine have been done on NNV challenged fishes previously and the relative percent survival (RPS) results were 72%, which indicates that NNVCP-S5E provides an effective prevention against NNV infection. However, the immune response characterized, whether TH1 or TH2 immune pathway is induced by vaccination of LAE vaccine in different temperatures are still unknown. Thus, juvenile giant grouper (*Epinephelus lanceolatus*) is used as a test subject in this project and the fishes were first acclimatized under three temperatures, 20°C, 28°C and 36°C for 1 week, and vaccinated through intraperitoneal injection (IP). Then, the fishes are challenged with purified NNV with a median lethal dosage, LD50 of 3.16.105copies/mL, and sacrificed to obtain immune related organs such. Real-time PCR (RT-PCR) is done in order to investigate the mRNA expressions of the TH1 or TH2 immune pathway markers, *T-bet* for TH1 pathway; *Gata-3* and *c-Maf* for TH2 pathway. Based on the RT-PCR analysis from all three groups, the gene expression results indicate that the vaccination tends to direct the fish immune response towards TH2 pathway of the adaptive immune response, which is a pathway towards the proliferation of B cells and a stronger antibody production. Furthermore, the study also shows a higher survival rate for the fishes that were acclimatized and vaccinated under 35°C compared to other temperatures after challenged with NNV for 14 days. This study contributed different insights which would help in a better protection against virus infection.

Keywords: Linear Array Epitope Vaccine, Grouper, Nervous Necrosis Virus, Temperature Difference, Immune Pathway

Corresponding author.

E-mail address: joannelam94@gmail.com (J.S.-W. Lam).

O-012.

Stress hormones modulate early immune activities in the head kidney of *Coregonus maraena*

J. Martorell-Ribera^{1, 3, #}, M. Nipkow¹, T. Viergutz², T. Goldammer¹, U. Gimsa³, A. Rebl¹.

¹Fish Genetics Unit, Institute of Genome Biology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany

²Service Group Cytometry, Institute of Reproductive Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany

³Psychophysiology Unit, Institute of Behavioural Physiology, Leibniz Institute for Farm Animal Biology (FBN), Wilhelm-Stahl-Allee 2, 18196, Dummerstorf, Germany

Abstract

Under challenging conditions including threat and discomfort, the vertebrate stress response triggers endocrine and neurologic networks. They release stress hormones such as cortisol and catecholamines in the head kidney. This endocrine and hematopoietic tissue is thus of central