



Disparate developmental patterns of immune responses to bacterial and viral infections in fish

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O-099.**Interaction of *Francisella noatunensis* subsp. *orientalis* with *Oreochromis mossambicus* Bulbus arteriosus cell line**Esteban Soto^{1,§}, Susan Yu¹, John Hansen²¹ University of California-Davis, Department of Medicine and Epidemiology, School of Veterinary Medicine, Davis, CA95616, USA² U.S. Geological Survey, Western Fisheries Research Center, Seattle, WA98115, USA

Francisella noatunensis subsp. *orientalis* (*Fno*) (syn. *F. asiatica*) is an emergent warm water fish pathogen and the causative agent of piscine francisellosis. Although *Fno* causes septicemia and can live extracellularly in a tilapia (*Oreochromis* spp.) infection model, the early interaction of *Fno* with vasculature endothelium is unknown. In the present study, we examined the interaction of wild-type *Fno* (WT) and two *Fno* knockout strains, intracellular growth loci C (Δ iglC) and pathogenicity determinant protein A (Δ pdpA), with a previously reported *O. mossambicus* Bulbus arteriosus endothelial-like cell line (TmB) at 25°C and 30°C. Similar amounts of WT, Δ iglC, and Δ pdpA attached and were detected intracellularly after 5 hours post-infection at both temperatures; however there was an effect of temperature on uptake as significantly greater quantities of *Fno* (WT, Δ iglC, and Δ pdpA) were detected intracellularly when cells were incubated at 30°C. Only the WT *Fno* was able to replicate intracellularly, causing cytotoxicity and apoptosis at 24 and 72 h post-infection when incubated at 25°C. WT *Fno* incubated at 30°C as well as Δ iglC, and Δ pdpA incubated at 25°C and 30°C were defective for survival, replication, and the ability to cause cytotoxicity in TmB. The current findings provide insight into the pathophysiology of francisellosis in tilapia.

Keywords: *Francisella*, endothelium, mutant, temperature, tilapia

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O-100.**Looking through transgenic zebrafish to reveal novel mechanisms of host-parasite interaction: In vivo real-time imaging of a trypanosome infection**Maria Forlenza[§], Eva Doro, Ffion Hammond, Pei Hongyin, Geert Wiegertjes

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Trypanosomes are unicellular flagellate protozoan parasites often with a preference for living in the blood of their hosts. *Trypanosoma carassii* naturally infects carp, goldfish and related species. Under normal conditions fish Trypanosomes do not cause major problems in wild stocks or aquaculture fish species, so why study them? The reason is twofold: 1) they are beautiful to look at under the microscope; 2) they can help reveal detailed immune mechanisms including, in vivo macrophage polarization, immune evasion strategies, B and T cell proliferation and memory responses. Over the past years our group has used a trypanosome infection model of carp as a tool to trigger and modulate the immune system and gain insight in fundamental protective mechanisms. To further expand our toolbox and overcome some of the limitations of the carp model, we established a trypanosome infection model of zebrafish, the little twin sister of carp. Through the use of larvae, juvenile and adult transgenic fish, we are able to: 1) visualize, in real-time, the mechanisms of parasite extravasation and leukocyte-trypanosome interaction; 2) visualize the kinetics of innate immune cell activation by using Tnfa:Gfp and Il1b:Gfp expressing transgenic zebrafish lines; and 3) investigate the role of macrophages in the resolution or exacerbation of infection through ablation experiments. Where the large carp is perfect for cellular studies, the small zebrafish is an excellent tool for live imaging and genetic manipulation. Together, they form a perfect 'twinning team' to

address fundamental questions on the evolution of the immune system and to unravel fundamental aspects of host-pathogen interaction.

Keywords: host-pathogen interaction, zebrafish, trypanosomes, immune response

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E-mail address: maria.forlenza@wur.nl (M. Forlenza).**O-101.****Disparate developmental patterns of immune responses to bacterial and viral infections in fish**Rosario Castro¹, Luc Jouneau¹, Luca Tacchi², Daniel J. Macqueen², Abdullah Alzaid², Christopher J. Secombes², Samuel A.M. Martin^{2,*}, Pierre Boudinot^{1,*}¹ Virologie et Immunologie Moléculaires, INRA, Jouy-en-Josas, France² Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, United Kingdom, AB24 2TZ**Abstract**

During early stages of development vertebrates rely on an immature immune system to fight pathogens. In humans, distinct phases of the development of immunity have been distinguished based on levels of TLR-induced cytokine responses. These periods are each associated with predominant infectious diseases reflecting the immaturity of responses. Whether such transitions during the development of immunity are conserved across vertebrates remains unknown. Here we examine the divergence between responses to bacterial and viral infections during early development in a teleost fish. Using rainbow trout, we characterized responses to two natural pathogens of this species, the Gram negative bacterium *Aeromonas salmonicida* and the virus VHSV, using microarray analysis at four early life history stages; eyed egg, post hatch, first feeding and three weeks post first feeding when adaptive immunity starts to be effective. All stages responded to both infections, but the complexity of the response increased with developmental stage. The response to virus showed a clear interferon response only from first feeding. In contrast, bacterial infection induced a marked response from early stages, with modulation of inflammatory, antimicrobial peptide and complement genes across all developmental stages. Whilst the viral and bacterial responses were distinct, there were modulated genes in common, mainly of general inflammatory molecules. This work provides a first platform to explore the development of fish immunity to infection, and to compare the age-dependent changes (from embryo to adults) across vertebrates.

Keywords: Development of immunity; rainbow trout; virus; bacteria; interferon

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O-102.**Effects of piscine reovirus infection on innate immune signalling in salmon**Mark Polinski^{1,§}, Julia Bradshaw¹, Sabrina Inkpen², Matthew Rise², Rosemarie Ganassin³, Kyle Garver^{1,*}, Stewart Johnson^{1,*}¹ Fisheries and Oceans Canada Pacific Biological Station, Nanaimo, Canada V9T6N7, British Columbia² Department of Ocean Sciences, Memorial University, St. John's, A1B3X9, Canada³ Vancouver Island University, Nanaimo, British Columbia V9R5S5, Canada