# ABSTRACTS

# ROLE OF IFN1 IN THE REGULATION OF THE RAINBOW TROUT ENDOGENOUS ANTIGEN PRESENTATION PATHWAY AT LOW TEMPERATURE

Quinn H. Abram[1], Tania Rodriguez Ramos[1], Niels C. Bols[1], Barbara A. Katzenback[1], and Brian Dixon[1]
[1]Department of Biology, University of Waterloo, Waterloo, ON. Canada

Low temperatures generally impair teleost immune responses, including the endogenous antigen presentation pathway (EAPP). In mammals, type I interferons (IFN), the cytokine mediators of the antiviral response, are known to regulate the EAPP. However, it is unknown if this is also the case in fish, and whether potential impairments in type I IFN expression at low temperatures might contribute to EAPP impairments. The rainbow trout hypodermal fibroblast cell line RTHDF was used as an *in vitro* model to study the effect of suboptimal temperature on the transcript levels and secretion of IFN1, a rainbow trout type I IFN, via qRT-PCR and quantitative ELISA respectively. At 4°C, both transcript level up-regulation and secretion of IFN1 were found to be delayed relative to 20°C following stimulation with poly(I:C). Furthermore, *in silico* analysis predicted the presence of interferon-stimulated response elements in the promoter regions of the pathway-specific members of the EAPP (*b2m*, *mhIa*, and *tapasin*) in rainbow trout, which should allow them to respond to IFN1 signalling. Transcript levels of these EAPP members were also measured following stimulation with poly(I:C), and their up-regulation was similarly delayed at 4°C. These results support the hypothesis that delayed IFN1 up-regulation could be involved in the impaired EAPP regulation at suboptimal temperatures, and suggest that rainbow trout antiviral responses might be impaired over the winter months. This would be problematic for Canadian aquaculture due to the potential for a greater incidence of viral infections during this time.

# INNATE IMMUNE RESPONSES OF RAINBOW TROUT INDUCED BY DOUBLE STRANDED RNA FORMULATED WITH PLANT DERIVED NANOPARTICLES

Tamiru Alkie[1], Jondavid de Jong[1,2], Kristof Jenik[1], Karl Klinger[2], Stephanie DeWitte-Orr[1]

[1]*Department of Health Sciences,* Wilfrid Laurier University**,** *Waterloo, ON*
[2]*Mirexus Biotechnologies, Guelph, ON*

The classical innate immune responses in vertebrates are initiated after the recognition of microbial molecular motifs such as double-stranded (ds)RNA by pattern recognition receptors (PRRs) that are constitutively expressed on host cells. This engagement between PRRs and pathogen-associated molecular patterns (PAMPs) triggers intracellular signaling pathways leading to the induction of effector molecules, including interferons and interferon stimulated genes (ISGs) contributing to pathogen clearance. In this study, a synthetic dsRNA, high molecular weight (HMW) polyinosinic:polycytidylic acid (polyI:C), was used as a model dsRNA molecule and was complexed with biodegradable nanoparticles (NPs). We assessed the effects of the dsRNA-NP complex at inducing antiviral effectors using a reporter cell line, RTG-P1, which expresses firefly luciferase under the control of the trout Mx1 promoter. Following this initial evaluation, innate immune responses to dsRNA-NPs or the free form of dsRNA were tested using rainbow trout gut cells (RTgutGC) in three culture scenarios: (1) in a monolayer (2) in 3D cultures on a porous transwell culture system and (3) in a coculture study with rainbow trout monocyte/macrophage cell line (RTS11). The results showed that RTgutGC efficiently internalized higher amounts of dsRNA-NPs complex compared to the free form of dsRNA. The complex induced significantly higher levels of IFN (IFN1) and ISGs (Mx1 and Vig3) transcripts in all *in vitro* culture systems tested that correlated well with protection of RTgutGC cells from infection by viral hemorrhagic septicemia virus strain IVa (VHSV-IVa). The data in the present study suggests an increased immunostimulatory potency of dsRNA delivered by novel NPs.

# FOUR STABLE CLASS I LINEAGES IN CARTILAGINOUS FISHES

Tereza Almeida[1,2], Ana Verissimo [1,3], Pedro Esteves [1,2], Yuko Ohta[4], Martin Flajnik[4]

*[1]CIBIO-InBIO, Centro de Investigacão em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, Universidade do Porto, Vairão, Porto, Portugal; [2]Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Porto, Portugal; [3]Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, Virginia, USA*
*[4]Department of Microbiology and Immunology, University of Maryland at Baltimore, Baltimore, Maryland, USA.*

**Abstract**
Chondrichthyes (sharks, rays and chimaeras) is the most basal vertebrate lineage possessing the basic features of the adaptive immune systems present in mammals, and thus is a key taxon to understand the emergence and evolution of vertebrate adaptive immunity. We will present novel results on the diversity of genetic lineages of the major histocompatibility complex (MHC) in Chondrichthyes. A new, MHC-linked nonclassical class I lineage (UDA) was found in all cartilaginous fishes, which is a single copy gene in Elasmobranchs but multicopy in Holocephalans. This new gene is apparently monomorphic and has a unique tissue distribution. The resulting protein is predicted to bind to a unique set of peptides in all Elasmobranchs rather than to allele-specific sets of peptides as is the case for polymorphic MHC classical class I molecules. At the cellular level, we found that UDA is expressed in gill predominantly at the luminal epithelium surface. Two other lineages of previously reported nonclassical class I genes in cartilaginous fishes were also examined in detail, of which one (UBA) is present in all species tested and is generally multicopy, while the other (UCA) is elasmobranch-specific and shows a wider gene number range. Our data suggest that early in vertebrate history there was a division of labor among MHC class I genes, most likely presenting antigens of different types to different subsets of T cells.

**INVESTIGATION OF RNA INTERFERENCE (RNAi) MEDIATED GENE SILENCING IN FISH**

Sarah K.W. Au and Stephanie J. DeWitte-Orr
Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, CANADA

RNA interference (RNAi) was first characterized in invertebrates and plants as an antiviral innate immune response. It is a natural antiviral defense mechanism to degrade viral RNA by virus-induced gene silencing. Studies show synthetic double stranded RNA (dsRNA) induces sequence-specific degradation of mRNA, resulting in gene silencing. RNAi based strategies to induce gene silencing are commonly employed in numerous invertebrates but have not been extensively used in fish. In this study, we will provide insights into whether fish cells (RTG-2 and EPC) are able to utilize dsRNA to induce RNAi and silence gene expression, as it does in plants and invertebrates. We performed *in vitro* experiments in both RTG-2 and EPC cell lines transiently expressing green fluorescent protein (GFP), adding dsGFP into the cell and measuring subsequent GFP intensity. In addition, we also targeted endogenously expressed RTG-2 host genes (IFN-1 and Myc), treating the cell with either dsIFN-1 *or* dsMyc dsRNA respectively and measuring mRNA levels by quantitative Real-Time PCR. This study will aid in a better understanding of the role of dsRNA in silencing gene expression in fish, with the goal of demonstrating its viability as a tool for an antiviral therapy, develop new drugs and vaccines to control infectious diseases.

# LONG-TERM, PROTEOME-SCALE ANALYSIS OF RAINBOW TROUT IMMUNE PROTEINS: IMPLICATIONS FOR AQUACULTURE VACCINE DEVELOPMENT

**F. K. Bakke[1], M. M. Monte[2], D. Causey[1], T. Cornulier[1], A. Douglas[1], D. Stead[3], S. A. M. Martin[1], D. J. Macqueen [4] and H. Dooley [5]**

*[1] School of Biological Sciences, University of Aberdeen, UK*

*[2] Laboratory of Immunology-Vaccinology, Faculty of Veterinary Medicine, University of Liège, Belgium.*

*[3]Aberdeen Proteomics, The Rowett Institute, University of Aberdeen, Aberdeen, UK.*

*[4] The Roslin Institute and Royal (Dick) Veterinary School, University of Edinburgh, UK.*

*[5]University of Maryland School of Medicine, Baltimore, MD. USA.*

## ABSTRACT

Infectious diseases pose a significant threat to the economic stability and expansion of finfish aquaculture. Vaccination is widely considered the best prevention strategy, but evaluation of immune protection typically relies on measuring immune gene expression at the mRNA level from terminally-acquired tissue samples. However, mRNA expression does not always correlate with tissue protein levels, providing an incomplete representation of the nature and kinetics of the immune response. In addition, inter-individual variation necessitates the use of large numbers of experimental animals to obtain sufficient statistical power. To overcome these limitations, we used a long-term, proteome-scale approach to identify and quantify changes in immune protein levels in rainbow trout (*Oncorhynchus mykiss*) plasma. These changes provide an indication of fish health and immune status, while also permitting non-lethal sampling. Although all experimental fish mounted an antigen-specific humoral response, the timing and magnitude of this, and the response trajectories of most immune-relevant proteins, differed markedly between individuals. However, certain immunological proteins were found to be more consistently expressed across all fish, and may represent useful biomarkers of the immune response. Together our data emphasise the importance both of judicious selection of immunological biomarkers, and of careful assessment of changes in the expression of such proteins over longer-term study periods, when considering whether or not an effective antigen-specific immune response has been mounted. More generally, this approach offers a useful tool to monitor fish immune responses, while dramatically reducing the number of experimental animals required.

# DELINEATING THE ROLE OF IMMUNE RESPONSE IN WHOLE BODY REGENERATION OF SEA STAR LARVAE

Katherine Buckley, Grace Wolcanski, Veronica Hinman

Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA

Regenerative abilities are wide-spread across metazoans, although the extent to which organisms are capable of regenerating varies widely across lineages. Although many animals are able to regrow specific cell types or limbs, the most extensive version is known as whole body regeneration. This phenomenon involves the coordinated re-growth of multiple cell and tissue types to reproduce complete body plans. A notable example of this is the larval stage of sea stars. Following bisection along the anterior-posterior axis, both larval halves are capable of complete regeneration. Additionally, larvae have potent healing responses and are able to recover from wounds within a few hours. Analysis of RNA-Seq data collected during larval regeneration indicates that this response consists of three components: wound healing, axis re-specification, and cell proliferation. Here, we will present data on how the larval immune system activates each of these arms.

# TRIM27-L INCREASES IFN-β SIGNALLING DOWNSTREAM OF dMAVS BUT NOT chMAVS

Lee K. Campbell and Katharine E. Magor

University of Alberta, Department of Biological Sciences, Edmonton, AB

Tripartite motif (TRIM) proteins have been shown to be potent antiviral proteins and can restrict replication of viruses such as influenza A virus. It is currently not known if these proteins can inhibit influenza in its reservoir host, the duck. Ducks show remarkable resistance to morbidity and mortality of viral infection when compared to other species such as chickens. TRIM27-L is a gene found in the MHC-B locus of ducks but appears to have been lost in chickens. TRIM27-L was previously shown to increase IFN-β downstream of RIG-I signalling, however it is currently not known where this protein interacts within this pathway or if it defends against influenza infection. TRIM27-L was found to increase IFN-β signalling when transfected with dMAVS however it decreased this signalling when cotransfected with chMAVS. As chMAVS and dMAVs only share 58% identity we hypothesized that TRIM27-L is interacting differently with MAVS proteins from different species. TRIM27-L was found to not be protective against infection when overexpressed in chicken fibroblasts and interestingly cells transfected with TRIM27-L were more susceptible to virus. However, when cotransfected with RIG-I, TRIM27-L restricts viral replication in chicken fibroblasts beyond that of RIG-I alone. In the future, we will investigate the differences between chMAVS and dMAVS modification by TRIM27-L and use mass spectrometry to determine if other proteins are interacting with TRIM27-L in this pathway. These studies will help our understanding of TRIM protein evolution and regulation of innate immune function.

**AN ANALYSIS OF THE ROLE OF SILVER NANOPARTICLES AS AN ANTIMICROBIAL AND IMMUNOSTIMULANT AGENT IN AQUACULTURE**

Mariela Cisco, Yang Ding, Yasuhiro Shibasaki, J. Oriol Sunyer

*Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, 19014*

Heavy use of antibiotics in aquaculture and the development of antibiotic resistance are the growing problems for food security and global health. Silver nanoparticles (Ag-NPs) have attracted immense attention as an alternative approach to control infectious agents. The aim of this study was to evaluate the inhibitory effects of Ag-NPs against distinctive fish pathogens, including one of the most detrimental parasites, *Ichthyophthirius multifiliis* (Ich), and two bacteria, *Flavobacterium columnare* (*F. columnare*) and *Yersinia rukeri* (*Y. rukeri*). These pathogens were exposed to Ag-NPs (particle size < 100 nm) at final concentrations of 0 (control), 1, 2, 10, 50, 100, and 250 μg/mL. Ag-NPs demonstrated significant inhibitory effects on Ich tomonts at 2 hours post cohabitation, as 63% to 87% of tomonts were killed by Ag-NPs of 1 to 100 μg/mL, compared to the 29% mortality rate observed in controls. Ag-NPs of 50 and 100 μg/mL could result in ~100% mortality of tomonts at 4 hours. ~100% mortality of the infective theronts was also observed after 4 hours post cohabitation with different concentrations of Ag-NPs. The antibacterial activity of Ag-NPs was also tested against *F. columnare* and *Y. rukeri*. Ag-NPs significantly inhibited the growth of *F. columnare* and *Y. rukeri* at concentrations of 100 and 250 μg/mL 24 hours post inoculation, respectively. We are currently evaluating the potential immunostimulatory action of Ag-NPs on rainbow trout immunity with the use of a panel of recently developed antibodies to trout immune cells and molecules, some of which were developed by the Immune Reagent Network for Aquacultured Species, funded by the US Department of Agriculture. Overall, our findings reveal strong antimicrobial properties of silver nanoparticles against the ectoparasitic Ich, as well as prevalent fish bacterial pathogens. In conclusion, we demonstrated that silver nanoparticles have significant promise in the aquaculture industry, opening new avenues of antimicrobial exploration for imminent cures and strategies. Future studies will allow the initial discovery to be confirmed and propelled.

# NONCLASSICAL LEUKOCYTE IMMUNE-TYPE RECEPTORS IN CHANNEL CATFISH, *ICTALURUS PUNCTATUS*.

Jonathan D. Crider, Laura Blackmon, Melanie Wilson, PhD, Eva Bengtén, PhD

*Department of Microbiology and Immunology, University of Mississippi Medical Center, Jackson, MS*

The Leukocyte Immune-Type Receptor (LITR) family consists of activating and inhibitory immunoregulatory receptors and is unique to teleosts. Family members vary in their number of immunoglobulin (Ig) domains, which are phylogenetically related to both Fc receptors and to receptors encoded within the leukocyte receptor complex. Previously, it was thought that all LITRs shared similar D1 and D2 Ig-domains, however we recently identified a subset that lacked these domains. One member of this nonclassical LITR subset, LITR622, contains eight Ig-domains beginning with a unique D1 that exhibits <42% amino acid identity with classical D1 domains. The LITR622 gene also encodes a distinct signal peptide and a D3 Ig-domain specific to this subset. Using a combination of Southern blot and genomic sequencing we predict there are 13 copies of this D3 exon in the catfish haploid genome. That a subset of CC41 mAb-reactive LITRs, expressed on catfish TS32.15 CTLs and NK cells, was upregulated in catfish during an anti-channel catfish virus (CCV) response, led us to investigate LITR622 gene expression in CCV-infected cells. Clonal G14D T cells were infected with CCV (MOI of 10) and total RNA was isolated from mock- and CCV-infected cells at 3hr, 5hr and 7hr post-infection. This time course confirmed that LITR622 gene expression was dependent upon viral replication, and poly(I:C) treatment also stimulated expression of LITR622. Such findings may indicate that some LITR622 proteins function as stress molecules which are recognized by certain "more classical" LITRs and further demonstrates the complexity of the LITR gene family.

# SHARK THYMUS EMPLOYS UNUSUAL ANTIGEN RECEPTORS AND IMMUNOGENETIC MECHANISMS

M.F. Criscitiello*[1], J.A. Ott[1], T.C. Deiss[1] [1]Texas A&M University, Department of Veterinary Pathobiology, College Station TX, USA mcriscitiello@cvm.tamu.edu

All jawed vertebrates appear to use the thymus for T cell development and shaping of the primary T cell repertoire. We study the immune system of the nurse shark as it represents the oldest class of animals with our immunoglobulin, T cell receptor (TCR), MHC and thymus based adaptive immunity. While much architecture and gene expression is conserved between shark and mammalian thymus, some interesting differences are emerging. While somatic hypermutation is generally considered a B cell phenomenon, shark thymocytes employ it to diversify some TCR chains, alpha to a significant degree. This appears concentrated at the cortico-medullary junction, begging questions of how positive and negative selection are influenced. TCR delta in sharks is capable of generating a few different forms. In addition to the canonical Vdelta repertoire there are doubly-rearranging NARTCR that have two V domains plus the delta C domain, and chimeric delta chains that use immunoglobulin heavy chain variable segments along with diversity and joining segments from delta to encode the variable domain. Some of these quirks of shark are also found in the T cell repertoires of other vertebrate groups, including mammals. We think the TCR immunogenetics of this shark model may offer insights into the genesis of the system and the plasticity available in engineering antigen receptor immunotherapeutics.

**The TNF superfamily ligand and receptor repertoire of cartilaginous fishes**

Rita Pettinello[1], Anthony K. Redmond[1,2], Helen Dooley[1,3]

[1]School of Biological Sciences and [2]Centre for Genome-Enabled Biology & Medicine, University of Aberdeen, Aberdeen AB24 2TZ, United Kingdom.[3]Dept. Microbiology & Immunology, University of Maryland School of Medicine, Institute of Marine & Environmental Technology (IMET), Baltimore MD 21202. USA.

The tumor necrosis factor superfamily (TNFSF) cytokines BAFF (TNFSF13b) and APRIL (TNFSF13) regulate mammalian B cell development, survival, and function through interaction with their shared receptors BAFF-R (TNFRSF13c), BCMA (TNFRSF17) and TACI (TNFRSF13b). BAFF is critical for the development and maintenance of antigen-naïve B cells, controlling the size and self-reactivity of the B cell pool. APRIL, in contrast, regulates the long-term survival of antigen-experienced plasma cells and homeostasis of peritoneal cavity B1 cells. Together these two ligands direct antibody-mediated protection in mammals. While completing a survey of the TNFSF and TNFRSF repertoires of cartilaginous fishes we identified orthologs of the receptors BAFF-R, BCMA, and TACI. In addition to the BAFF orthologue previously identified by our group, we also identified an APRIL orthologue in cartilaginous fishes. Unexpectedly, we also found a third TNFSF13-family ligand, BALM (proposed TNFSF13c), previously thought to be a bony fish-specific TNFSF member. A comprehensive phylogenetic analysis, incorporating the new cartilaginous fish sequences, reveals that an APRIL-like molecule was most likely the founding member of the TNFSF13 family, being present in the ancestor of all vertebrates. Further, that BALM was present when immunoglobulin-expressing B cells emerged in the ancestor of jawed vertebrates; while retained by all extant fishes (cartilaginous, bony, and lobe-finned), BALM was secondarily lost in the common tetrapod ancestor. Our results will be discussed in the context of vertebrate B cell evolution.

# SEASONAL CHANGES IN THE NORTH AMERICAN WOOD FROG SKIN MICROBIOME

Alexander J. Douglas, Laura A. Hug, Barbara A. Katzenback

*Department of Biology, University of Waterloo, Waterloo, ON, Canada*

Recent research has highlighted the importance of the frog skin microbiome in innate immune defence, notably that some commensal bacteria produce anti-fungal secondary metabolites effective against *Batrachochytrium dendrobatidis*. North American wood frogs (*Rana sylvatica*), native to Canada and the Northern United States, are freeze-tolerant animals that breed in vernal pools upon spring thaw and spend the remainder of the year inhabiting the forest floor. Given than wood frogs are susceptible to *B. dendrobatidis* and Frog Virus 3, the objective of our research was to characterize the wood frog skin microbiome and potential variations in microbial composition across seasons. During the spring, summer and fall months, frogs were captured, and their dorsal and ventral surfaces swabbed. Pond water samples were taken during the spring. Total DNA was extracted and the V4 barcode region of the bacterial 16S rRNA gene was sequenced (Illumina MiSeq). Sequences were analyzed using QIIME2, assigned taxonomy, and assessed with α- and β-diversity metrics. Results suggest that *R. sylvatica* skin hosts a unique microbiome independent of the pond they inhabit. Characteristics such as sex and pond of origin did not relate to trends in the microbiome, but significant seasonal variance was observed. Cyanobacteria were proportionally much more abundant during spring, while actinobacteria and acidobacteria were better represented in the summer and fall. Further investigations into the roles of the microbial community are required to better understand the functional significance of seasonal variation in the skin microbiome and its contribution to defence against pathogens.

# Temperature impacts on Atlantic Salmon (*Salmo salar)* response to infection with *Renibacterium salmoninarum*

Jonathon E. Perreira, Sara L. Purcell, and Mark D. Fast.

Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE C1A 4P3, Canada.

Mitigating the impact of climate-related challenges on salmon and understanding how climate factors affect host-pathogen interactions is essential to the future sustainability of salmon aquaculture. Among these challenges, is understanding the mechanisms by which current pathogens, like *Renibacterium salmoninarum* (Rsal), exploit their hosts, Atlantic salmon (*Salmo salar*), and under what conditions the host is able to fight off infection and prevent the development of Bacterial Kidney Disease. For most of the year in Eastern North American sea cage culture, temperatures are between 8-13°C, however, IPCC and other future global climate predictions foresee coastal regions experiencing extended periods up to and beyond 20 °C. For this reason, multiple studies have been initiated to investigate BKD progression and Atlantic salmon immune responses at 13 °C (i.e. current temperature) compared to 20 °C (i.e. future temperature). Following a period of acclimation to these different temperature regimes, salmon smolts (150-300 g) were inoculated (i.p.) with a culture of *R. salmoninarum*. Over multiple studies, mortalities at 13 °C reached between 50-100%, whereas exposure at 20 °C resulted in 0% mortality. Despite, Rsal growth in culture at 20 °C no clinical signs were observed in salmon exposed at 20 °C, but also showed no significant difference in survival upon re-exposure at 13 °C (ca. 50%), compared to naïve salmon exposed at 13 °C (ca. 50%). Dual transcriptome analysis of head kidneys of exposed Atlantic salmon showed impacts in endo/exocytosis, phagosome, lysosome, MAPK signaling, NOD-like receptor signaling and other pathways associated with intracellular pathogen recognition and processing. Temperature impacts and exposure history will be discussed with respect to antibody production, recognition and intracellular success of Rsal infection in Atlantic salmon.

# EXAMINATION OF CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) LEUKOCYTE IMMUNE-TYPE RECEPTOR (IPLITR)-MEDIATED CROSS-TALK REGULATION OF PHAGOCYTOSIS

Chenjie Fei, Myron Zwozdesky, Dustin Lillico, Luke Gerla, and James L. Stafford
Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada.

Channel catfish leukocyte immune-type receptors regulate various innate immune cell responses via classical and unique intracellular signaling networks. We have shown IpLITR 2.6b/IpFcRγ-L activates immune cell effector responses using a canonical ITAM-dependent signaling pathway whereas IpLITR 1.1b is an inhibitory receptor due to ITIM-mediated phosphatase recruitment. Since both stimulatory and inhibitory IpLITR-types are co-expressed by catfish immune cell-types, integrated signaling between these proteins (i.e. cross-talk) are likely important for their overall immunoregulatory functions. Known IpLITRs also share similar extracellular regions suggesting that they may engage common ligands, however detailed mechanisms regarding potential cross-talk between different IpLITR-types remain unknown. Here we generated AD293 cell lines co-expressing the N-terminal HA-tagged IpLITR 2.6b/IpFcRγ-L proteins and a FLAG-tagged IpLITR 1.1b construct. This allowed co-engagement of the receptors with mAb-coated microbeads and development of an imaging flow cytometry-based phagocytosis cross-talk assay. Our results show that IpLITR 1.1b inhibits IpLITR 2.6b/IpFcRγ-L-mediated phagocytic response. Induction of phosphotyrosine signals downstream of IpLITR 2.6b/IpFcRγ-L activation was significantly reduced when co-engaged with IpLITR 1.1b, likely through the activation of Src homology region 2 domain-containing phosphatase-2 (SHP-2). Additionally, two tyrosine motifs (i.e. a Csk-binding motif and an ITIM) in the cytoplasmic region of IpLITR 1.1b were shown to be critical for sustaining the inhibition of phagocytosis, providing new information about coordinated recruitment of effector molecules (e.g. Csk and SHP-2) in regulating phagocytosis. Overall, this work reveals that co-engagement of IpLITRs can fine-tune the balance between activating and inhibitory signals, which governs the overall control of a vital innate cell effector response.

**Validation of an Interleukin-1 Beta Assay for Assessing Immune Function in Salmonids**

Aaron Frenette1, Tania Rodríguez-Ramos1, Shawna Semple1, Fabio Zanuzzo2, Cheryl Soulliere1, Jack Iwanczyk3, Jessi Rix4, Devyn Ramsay2, Kurt Gamperl2 and Brian Dixon1

1Department of Biology, University of Waterloo, Waterloo, ON, 2Department of Ocean Sciences, Memorial University of Newfoundland, St. John's, NL, 3Cedarlane Laboratories Limited, Burlington, ON, 4Somru BioScience Inc., Charlottetown, PE

Salmonid aquaculture in Canada faces several climate change-related challenges, including increasing water temperatures and hypoxic events, which may exacerbate bacterial and viral diseases. However, functional (and quantitative) immunological tools are needed if the industry is to select efficacious vaccines/treatments and to improve fish health/disease management, and we are to better understand how the fish immune system responds to such challenges. Genes encoding small signaling proteins (cytokines) that regulate immune/stress responses have been identified in many fishes, but their biological function(s) have not been elucidated. Herein, we report on the use of a quantitative enzyme-linked immunosorbent assay (ELISA) to measure interleukin-1 beta (IL-1β) in rainbow trout (*Oncorhynchus mykiss*). IL-1β gene transcript levels, measured by qPCR, were consistent with an increase in IL-1β protein production/secretion in both cell culture (monocyte/macrophage cell line) and in primary spleen cells stimulated with heat-killed *Vibrio anguillarum*. *In vivo* assessment of the response of trout to acute heat-stress revealed that the expression of IL-1β transcripts in whole blood was consistent with the secretion of IL-1β protein in the plasma. In addition, assay validation was demonstrated by being able to identify putative IL-1β precursor and mature versions from fish tissues and cell cultures using Western blots. This research validates the use (effectiveness) of this assay, and suggests that the biological function(s) of IL-1β may be analogous to the mammalian paradigm for signaling and activation.

**n=226 words**

# TRANSCRIPTOMIC PROFILING OF *STRONGYLOCENTROTUS PURPURATUS* COELOMOCYTE POPULATIONS

Fu-Yi Hsieh[1], Jun Fang Cheng[2], and Hsiao Han Huang[2], Katherine M. Buckley[3], Sebastian D Fugmann[1,2,4,5].

[1]Division of Microbiology, Graduate Institute of Biomedical Sciences, Chang Gung University, Kwei-Shan District, Tao-Yuan, Taiwan, [2]Department of Biomedical Sciences, Chang Gung University, Kwei-Shan District, Tao-Yuan, Taiwan, [3]Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, [4]Chang Gung Immunology Consortium, Chang Gung Memorial Hospital and Chang Gung University, Kwei-Shan District, Tao-Yuan, Taiwan. [5]Department of Nephrology, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan

Coelomocytes are important cellular mediators of immunity in echinoderms. In the purple sea urchin (*Strongylocentrotus purpuratus*) these cells can readily be divided into phagocytes, vibratile cells, red spherule cells, and colorless spherule cells based on their morphology. While some information has been reported about genes that are expressed in specific types of coelomocytes, a comprehensive analysis of the gene expression pattern in each cell type was not available. Using a density gradient coelomocytes from individual adult urchins were separated into three populations (phagocytes, a mixture of vibratile and colorless spherule cells, and red spherule cells), RNA was isolated, and next generation sequencing was employed to determine the transcriptome of each cell population. All three cell populations showed clear differences in their gene expression patterns, and the differences in transcript levels for selected genes that were found to be uniquely expressed in one cell type were confirmed by qRT-PCR using cDNA obtained from additional sea urchins. We now complemented this bulk population data with single-cell RNAseq to define coelomocyte identity and function based on their gene expression profiles. The new findings from this study will be discussed.

This work was supported by the Chang Gung Memorial Hospital (CMRPD1C0242, CMRPD1F0421, and CORPD1F0051) and the Ministry of Science and Technology Taiwan (MOST 107-2320-B-182-002)

# *BIOMPHALARIA GLABRATA* GRANULIN GRANTS HEIGHTENED LEVELS OF RESISTANCE TO *SCHISTOSOMA MANSONI* INFECTION BY INDUCING HEMOCYTE PROLIFERATION AND REACTIVE OXYGEN SPECIES PRODUCTION

Abdullah A. Gharamah[1], Jacob R. Hambrook[1], Emmanuel Pila[1], Solomon Hussein[2], Patrick C. Hanington[1]

[1]School of Public Health, University of Alberta, [2]Biological Sciences, University of Alberta

## Abstract

Granulins are an evolutionarily conserved family of growth factors present in all eukaryotic species, excluding fungi. These proteins serve as growth factors that can elicit cellular replication and differentiation; however, pro-granulins can be cleaved by elastase to generate functionally distinct granulin fragments that have been shown to be immunostimulatory. Gastropod mollusks, which have co-evolved with parasitic digenean trematodes for millions of years, utilize circulating heamocytes as the primary method of containing and killing these invading parasites. Here, we present the functional characterization of *Biomphalaria glabrata*'s granulin (BgGRN) during *Schistosoma mansoni* challenge. We demonstrate an increase in BgGRN transcript abundance in both *S. mansoni*-resistant and susceptible strains of *B. glabrata* following *S. mansoni* challenge. Addition of recombinant (r) BgGRN elicits haemocyte replication, with newly replicated cells frequently expressing BgTLR, a marker of resistance to *S. mansoni*. Additionally, cleaved rBgGRN subunits are capable of eliciting the release of Reactive Oxygen Species (ROS) from haemocytes *in vitro*. Injection of rBgGRN into susceptible snails reduced infection prevalence, while siRNA knock down of BgGRN resulted in increased susceptibility in typically resistant strains. This marks the first functional characterization of an endogenous growth factor of a gastropod mollusc, and is also the first gain-of-resistance study in a snail-digenean infection model using a defined factor to induce snail resistance to infection.

# MACROPHAGE DIFFERENTIATION CRITICALLY DEFINES SUSCEPTIBILITY AND RESISTANCE TO MYCOBACTERIA: NEW INSIGHTS FROM AN AMPHIBIAN MODEL

Milan Popovic[1], Amulya Yaparla[1], Dominic Paquin-Proulx[2], Daphne V. Koubourli[1], Rose Webb[3], Marcia Firmani[4], Leon Grayfer[1]

[1]Department of Biological Sciences, [2]Department of Microbiology, Immunology & Tropical Medicine, [3]Pathology Core Laboratory, [4]Department of Biomedical Laboratory Sciences, George Washington University, Washington, DC 20052, USA

*Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis (TB), remains the leading global cause of death from an infectious agent. Mycobacteria thrive within their host macrophages (Mϕs) and presently there is no animal model that permits combined *in vitro* and *in vivo* study of mycobacteria-host Mϕ interactions. *Mycobacterium marinum* (*Mm*), which causes tuberculosis in aquatic vertebrates, has become an auspicious model for TB research, owing to its close genetic relatedness to *Mtb* and the availability of alternative, natural host aquatic animal models. We adopted the *Xenopus laevis* frog-*Mm* surrogate infection model to study host Mϕ susceptibility and resistance to mycobacteria. Mϕ differentiation is regulated though the colony-stimulating factor-1 receptor (CSF-1R), which is activated by CSF-1 and the unrelated interleukin-34 (IL-34) cytokines. Using combined *in vitro* and *in vivo* approaches, we demonstrated that CSF-1-Mϕs exacerbate *Mm* infections, are more susceptible to mycobacterial entry and are less effective at killing this pathogen. By contrast, IL-34-Mϕs confer anti-*Mm* resistance *in vivo*, are less susceptible to *Mm* entry and more effectively eliminate internalized mycobacteria. Moreover, we showed that the human CSF-1- and IL-34-Mϕs are likewise respectively susceptible and resistant to mycobacteria, and that both frog and human CSF-1-Mϕs are more prone to the spread of mycobacteria and to being infected by *Mm*-laden Mϕs than the respective IL-34-Mϕ subsets. This work marks the first report describing the roles of these Mϕ subsets in mycobacterial disease and may-well lead to the development of more targeted anti-*Mtb* approaches.

# EXPLORING THE POTENTIAL BENEFITS OF THERMAL PRECONDITIONING ON THE ANTI-VIRAL IMMUNE RESPONSE OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

Huming Guo[1], Lindy Whitehouse[1], Moira Ferguson[2], Roy Danzmann[2], Brian Dixon[1]

[1]Dept. of Biology, University of Waterloo, Waterloo, ON. Canada

[2]Dept. of Integrative Biology, University of Guelph, Guelph, ON. Canada

The salmonid-dominated aquaculture industry, worth approximately $1 billion in Canada, is a steadily growing subsector of the Canadian economy. However, the industry is currently experiencing significant losses of revenue due to environmental stressors such as rising water temperatures and viral diseases. Water temperatures deviating from the salmonid's thermal optimum can exacerbate the spread of viral activities, and the current lack of effective vaccinations calls for additional prevention methods that can help fish withstand viral pathogens. In this study, we evaluated the potential benefits of thermal preconditioning on the rainbow trout anti-viral response. Juvenile fish (four months post-fertilization) were preconditioned in supra-optimal water temperatures (18$^{o}$C - 19$^{o}$C) twice per week, for six weeks. Spleen samples were collected from these preconditioned fish during a thermal stress challenge 16 weeks after the end of the preconditioning. The thermal stress challenge entailed subjecting the preconditioned and non-preconditioned cohorts to water temperatures raised from 12$^{o}$C to 28$^{o}$C at a rate ~2 $^{o}$C per hour. Individuals were collected and sampled at the time of lost equilibrium. IFN-1, MHC class I and β2m transcript levels in the collected spleens of preconditioned and non-preconditioned cohorts were assessed with qPCR to examine the modulation of the anti-viral response in low tolerance, high tolerance, and control fish. The outcomes of this study could identify potential benefits of thermal preconditioning in strengthening the immune defenses of salmonids against viral pathogens.

**IDENTIFICATION, MOLECULAR CHARACTERIZATION, AND EXPRESSION ANALYSES OF ZEBRAFISH (*Danio Rerio*) LEUKOCYTE IMMUNE RECEPTOR-TYPES**

Hima Gurupalli, Chenjie Fei, Myron Zwozdesky and James Stafford

Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada

Innate immune cells sense and respond to external environmental cues using cell surface-expressed receptors that trigger intracellular signalling cascades to generate specific effector responses. The channel catfish (*Ictalurus punctatus*) leukocyte immune-type receptor (IpLITR) family consists of multiple receptor-types with variable signaling abilities. Using *in vitro* approaches, our work has shown that IpLITRs are potent regulators of various innate immune cells responses including degranulation, cytokine secretion, and phagocytosis. To better understand the potential roles of LITRs *in vivo*, this study focused on using zebrafish as a model organism to further examine teleost LITRs. Two putative Danio rerio (Dr)LITRs, DrLITR 1.1 and 1.2, were identified in the zebrafish genome. The BLASTp results suggested that these receptors are closely related to mammalian FcRLs and CD22. DrLITRs 1.1 and 1.2 contain 3 immunoglobulin (IG) domains, a transmembrane region and a cytoplasmic tail containing both immunoreceptor tyrosine-based activation motif (ITAM) and immunoreceptor tyrosine-based inhibition motif (ITIM). We cloned, sequenced and analyzed the expression of these two receptors throughout ontogeny and adulthood with and without an immunostimulant. Our results showed that DrLITRs 1.1 and 1.2 are expressed as early as 1 hour post fertilization (hpf) and remained expressed throughout embryonic development and adulthood. Although TNFα, IL-8, and IL1β, displayed significant increases in expression using a visceral cavity based inflammation assay, DrLITR 1.1 and 1.2 expression was unchanged over the same period following intraperitoneal injection with 1µg/mL Zymosan. Overall, this work sets the stage for establishing zebrafish as a model system to study these novel immunoregulatory receptors.

# DIFFERENTIAL BEHAVIOURAL THERMOREGULATION RESPONSES OF TELEOST FISH

Farah Haddad [1], Shelby Gordychuk [1, ‡], Susana Thiessen [1, ‡], Daniel R. Barreda [1, 2]

[1] *Department of Biological Sciences, University of Alberta*

[2] *Department of Agricultural, Food and Nutritional Sciences*

[‡] *Contributed equally to this work*

Fever is a hallmark of acute inflammation, widely conserved for over 500 million years of metazoan evolution. It is characterized by a short-term (hours to days) increase in body temperature. Despite its long-standing association with acute inflammation, little is known about the effects of fever on immunity. Aquatic animals such as fish have been previously shown to select warmer temperatures upon infection, and this offers a survival advantage. Our Aim was to define the teleost behavioural fever response following an immune challenge, and to assess whether specific responses are activated against different immune challenges . A zymosan-induced peritonitis model and a live *Aeromonas veronii* infection model were used to study the behavioural response using a high-resolution custom setup. We found that zymosan challenge led to an increased in temperature preference simultaneously with two new lethargy behaviours: a) a decrease in swimming velocity, and b) migration rates across different thermal zones. These results were paired with an increased efficiency of leukocyte recruitment into the immune challenge site compared to control fish held at standard housing temperatures, early expression of pro-inflammatory cytokines, and promotion of antimicrobial response. The behavioural thermoregulatory response against *A. veronii* was distinct from that against zymosan and led to rapid clearance of *Aeromonas*. These findings suggest important benefits of fever in fish facing pathogen infections.

# *SCHISTOSOMA MANSONI MODULATES THE IMMUNE RESPONSE OF BIOMPHALARIA GLABRATA USING A LEISHMANOLYSIN-LIKE MATRIX METALLOPROTEASE*

Jacob R. Hambrook[1], Alethe Kabore[1], Emmanuel Pila[1], Patrick C. Hanington[1]

[1] School of Public Health, University of Alberta

An estimated 200 million people worldwide suffer from schistosomiasis which, causes approximately 200,000 deaths annually. *Schistosoma mansoni*, the species responsible for the majority of infections, utilizes snails of the genus Biomphalaria to complete its larval development. Whether *S. mansoni* is able to establish a successful infection is predicated on evading or avoiding the snail immune response, which involves encapsulation of the parasite and subsequent killing by snail hemocytes or soluble immune effectors. How *S. mansoni* evades this immune response response is not well understood, however, it is known to be driven by *S. mansoni* excretory/secretory (E/S) products. We have identified and characterized a matrix-metalloprotease (SmLeish) found within these E/S products. This protein is present in high abundance at several key *S. mansoni* life cycle stages and it exists as a membrane bound protein that is subsequently cleaved into a soluble form during the initial stages of the snail infection. SmLeish is present in close association with developing sporocysts *in vivo* and functional analyses using a recombinant *Sm*Leish confirm that it possesses classic metalloprotease activity. The rSmLeish inhibits hemocyte movement, which is also a property of E/S products. Knock down of *Sm*Leish increases sporocyst encapsulation rates *in vitro* while also resulting in lowered infection kinetics within susceptible *B. glabrata* snails, as well as lowered cercarial output from successfully infected snails. These findings clearly demonstrate that *Sm*Leish plays a role in immune interference of the snail host during sporocyst development, while additionally providing insight into the mechanism by which this immune regulation occurs.

# TRAINING THE CHICKEN INNATE IMMUNE SYSTEM

Wanwei He1, Daniel R. Barreda1,2
1Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada
2Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, Alberta, Canada

Inspired by the newly discovered capacity of memory established in innate arm of immunity, termed trained immunity, we aim to establish an antibody independent and drug free prevention strategy against Necrotic Enteritis(NE) in chicken. Opportunistically caused by *Clostridium perfringens*, part of the intestine commensal flora, NE has casted great challenges in disease control. Current challenges include underdeveloped adaptive immunity in neonatal birds, the decline of maternal antibody by week 3, and the tendency of immune response moving from rejection towards tolerance against commensal bacteria. β-glucan is a well-studied pathogen-associated molecular patterns (PAMPs) promoting innate protection via trained immunity, has been demonstrated to reverse the established tolerance phenotype in monocytes. This mechanism also reprograms cell differentiation and cytokine activity for a stronger rejection response. We have identified increased robustness in leukocyte infiltration dominated by heterophils, ROS production within monocytes/macrophages, and higher expression of TNF-α, IL-1β and CXCL-8 following intra-abdominal β-glucan injection. Our next step is to assess the contributions of this PAMP on trained immunity against *C.perfringens*, by introducing β-glucan to animals during first contact with the pathogen. We are currently evaluating this using a natural infection model where opportunistic infection results from intestinal dysbiosis. We expect to observe a stronger pro-inflammatory antimicrobial response based on immune cell infiltration, activation status, phagocytosis and cytokine production, leading to reduced intestinal *C.perfringens* burden and disease severity in the trained animals.

# PATHOGEN SPECIFIC ANTIBODY TITRES AMONG SEVEN OUTBRED CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*) POPULATIONS AND THEIR RELATIONSHIP TO SURVIVAL RATES DURING PATHOGEN CHALLENGE

George Heath[1], Shawna L. Semple[1], Daniel Heath[2] and Brian Dixon[1]

[1]Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, ON N2L 3G1
[2]Great Lakes Institute for Environmental Research, University of Windsor, 2601 Union Street, Windsor, ON N9B 3P4

Aquaculture in Canada suffers significant losses due to disease, estimated at ~35% of the animals annually at Canadian latitudes. This translates into approximately $500 million annually for the Canadian aquaculture industry Outbreeding programs intended to generate hybrid vigor are an important aspect of combatting this problem. In this study inbred Chinook salmon were outcrossed with populations originating from seven different rivers in the lower mainland of BC and Vancouver Island. The offspring were used in an infection challenge with *Vibrio anguillarum*, the results of which indicated differential susceptibility to the pathogen across different populations. A subtractive indirect ELISA was used to quantify circulating levels of *Vibrio* specific antibodies in the plasma of different populations. Antibody levels generally increased in infected fish over the course of the 28 day trial period, with different populations showing variable responses. One of the two most robust families displayed high antibody titre at 28 days post injection however this was also the case for one of the two most susceptible families. Analysis of major histocompatibility alleles present in each population indicated that farmed salmon had different degrees of heterozygosity than their wild counterparts. The results suggest that outbreeding may have beneficial effects for the Canadian aquaculture industry and could help prevent costly disease losses.

SHANEE L. HERRINGTON-KRAUSE, S.J. POYNTER AND S.J. DEWITTE-ORR
Department of Biology, Wilfrid Laurier University, Waterloo ON. Canada.
email: herr0600@mylaurier.ca

# Exploring the antiviral mechanisms of rainbow trout vig-3

# Exploration du mécanisme antiviral de la truite arc-en-ciel vig-3

Rainbow trout is the most farmed fish in Ontario and pathogen infections, including virus infections, cause catastrophic mortality rates and substantial economic losses. Despite this, there is a lack of understanding regarding fish innate immunity, specifically with regards to interferon-stimulated genes (ISGs) and their antiviral effects. Viral Hemorrhagic Septicemia Virus (VHSV) induced gene (vig)-3 in rainbow trout is homologous to ISG15 in mammals. It is a small ubiquitin-like protein inducible by type I interferons (IFN), suggested to have antiviral effects within the cell. Vig-3's antiviral activity is proposed to act both intracellularly through covalent modification of proteins, as well as extracellularly as a signaling molecule. The proposed project aims to investigate the role of vig-3 in rainbow trout antiviral innate immunity. To do this, rainbow trout gonadal cells (RTG-2) will be infected with fish viruses, and the expression of vig-3 expression will be monitored over the course of each infection. The subcellular localization of vig-3 will be observed via immunocytochemistry during viral infection, and the ability of overexpressed vig-3 to limit virus infection will be investigated. These findings will contribute to a better understanding of a poorly studied aspect of innate antiviral immunity in an economically valuable fish species.

# The immune response in sea urchin *Strongylocentrotus purpuratus* has dynamic changes in immune cell populations

Megan A. Barela Hudgell, Leon Grayfer, & L. Courtney Smith
Department of Biological Sciences, George Washington University, Washington, DC 20052, USA

The sea urchin, *Strongylocentrotus purpuratus*, has four morphologically distinct coelomocyte types including phagocytes, red spherule cells, colorless spherule cells, and vibratile cells. The total sea urchin coelomocyte numbers are known to increase in response to pathogen challenge. However, changes within each respective coelomocyte sub-population in response to immune challenge remain largely unknown. Accordingly, we developed a gating strategy for flow cytometry to distinguish distinct coelomocyte sub-populations including large phagocytes, small phagocytes, red spherule cells, and a mixed population of vibratile cells and colorless spherule cells. Coelomocytes from immunoquiescent sea urchins were collected pre-challenge to characterize the cell populations, and subsequently evaluated for responses to needle puncture, and withdrawal of coelomic fluid. These animals were injected twice on days 2 and 5 with either heat killed *Vibrio diazotrophicus* (n = 8), zymosan A (n = 3), or artificial coelomic fluid (aCF, negative control, n = 7). Cell populations were evaluated on days 3 and 6 to characterize responses at 24 hours after injections with *Vibrio,* zymosan, or aCF. *Vibrio* induced an increase in total coelomocyte concentration, while aCF injection induced a decrease. *Vibrio* challenge significantly increased the concentration of large phagocytes while increasing both the concentration and proportion of red spherule cells, whereas zymosan significantly increased the concentration of small phagocytes. These results demonstrate that the sea urchin immune cell populations undergo dynamic changes *in vivo* in response to injury and to contact with distinct foreign particles.

KRISTOF JENIK1, T.N. ALKIE1, EMILY MOORE2, LEJ LEE3 and S.J. DEWITTE-ORR1
1 Dept. of Biology, Wilfrid Laurier University, Waterloo ON. Canada.
2 Mirexus Biotechnologies, Guelph ON
3 Faculty of Science, University of the Fraser Valley, Abbotsford BC. Canada
Jeni8010@mylaurier.ca

# USING A NOVEL NANOPARTICLE TO ENHANCE INOSINE MONOPHOSPHATE DELIVERY IN BOVINE AND RAINBOW TROUT CELLS

Animal health is essential for profitable animal husbandry, as healthier farmed animals are more capable of defending themselves against infectious diseases. In the past, nutrition has been a key strategy to support and maintain good animal health. The purpose of the present project is to increase bioavailability of inosine monophosphate (IMP), a key micronutrient for two important Canadian farmed animals, Alberta cattle and Ontario rainbow trout, in order to increase growth rates and strengthen immune responses. Increased bioavailability will be achieved by binding inosine monophosphate (IMP) to a novel, proprietary nano-carrier (NC) that can effectively enter cells. To date, IMP-NC has been more effective than IMP alone at enhancing cellular metabolism in the rainbow trout intestinal cell line (RTgutGC), but not in the bovine intestinal myofibroblast cell line (BT-IMF). Both cell lines demonstrated enhanced proliferation with IMP-NC treatment but not IMP alone. Interestingly, in RTgutGC IMP-NC also induced a prolonged innate antiviral immune response as measured by qRT-PCR. As BT-IMF is a novel cell line, characterization of the cell line is also included in the project. This work forms a basis for future in vivo studies, to enhance fish and cow growth using nano-carrier technology and IMP.

# GENERATION OF BONE MARROW CELL LINES FROM XENOPUS LAEVIS AND RANA SYLVATICA AND CHARACTERIZATION OF THEIR SUSCEPTIBILITY TO FROG VIRUS 3

Bibi S. H. Sokeechand, Mercedes M. Rose, William A. Ng, Maxwell P. Bui-Marinos, and Barbara A. Katzenback

*Department of Biology, University of Waterloo, Waterloo, ON, Canada*

In frogs, the bone marrow is the site of hematopoiesis and this process is supported by cellular and soluble signals provided by stromal cells within the hematopoietic niche. In susceptible frog species, such as the North American wood frog (*Rana sylvatica*), the bone marrow is one of the sites of Frog Virus 3 (FV3) replication and can lead to destruction of the hematopoietic niche. To better understand the role of bone marrow stromal cells in frog hematopoiesis and in host-virus interactions, we generated bone marrow cell lines from adult *Xenopus laevis* (Xela BMW3-1) and *R. sylvatica* (Rs BM7) using the tissue explant method. Both cell lines are adherent, stromal-like and exhibit low levels of senescence-associated β-galactosidase senescence. Xela BMW-3-1 and Rs BM7 are maintained at 26°C and 18°C, respectively, in amphibian-adjusted Leibovitz's L-15 medium supplemented with 15% FBS and 25% cell-conditioned media from previous cultures. Cells were infected with FV3 at 0, 0.002, 0.02, 0.2, 2 and 20 multiplicities of infection and monitored over 14 days to observe cellular morphology and viral replication. Xela BMW3-1 and Rs BM7 were susceptible to FV3 infection, exhibiting dose- and time-dependent cytopathic effects (loss of adherence) and increases in viral titre. These results suggest that bone marrow stromal cells from relatively FV3-resistant (*X. laevis*) and FV3-susceptible (*R. sylvatica*) frog species are equally permissive to FV3 entry and replication. Further studies are needed to characterize the growth factors produced by frog bone marrow stromal cells and cellular immunocompetence.

**Excretory-Secretory Products from the Entomopathogenic Nematode *Heterorhabditis bacteriophora* Enhance the Susceptibility of *Drosophila* to Bacterial Infection via Suppression of the Imd Immune Signaling Pathway.**

Eric Kenney[1], Jonathan Vadnal[1], John M. Hawdon[2], Damien O' Halloran[1], and Ioannis Eleftherianos[1].
Department of Biological Sciences[1], Department of Microbiology, Immunology, and Tropical Medicine[2] The George Washington University, Washington, DC.

In order to promote the success of an infection, parasitic helminths are known to secrete factors that manipulate the host immune system. A variety of applications await the identification of these factors, including the enhancement of entomopathogens used for the biocontrol of insect pests and the counter-suppression of vertebrate-infective helminths, but prerequisite to this identification is a foundational understanding of the total immunomodulatory capacities of the parasite. To this aim, infective juveniles (IJs) of the entomopathogen *Heterorhabditis bacteriophora* were activated in insect hemolymph before bulk secretions were collected and concentrated. Secreted products were then injected into *Drosophila melanogaster* flies, revealing through qPCR measurements distinct suppression of the Imd pathway, which serves as the primary signaling mechanism for coordinating an immune response against gram-negative bacteria. In conjunction with observations that the Toll pathway is not affected by the injection of secretions and that secretions from non-activated nematodes significantly increase Imd-based expression, the Imd pathway can be implicated as a primary axis of the immune response against a helminth infection. Furthermore, co-injection of the secreted products along with gram-negative bacteria enhanced the susceptibility of flies to bacterial infection, despite the tendency of activated secretions to enhance phagocytic activity in flies, as measured by pHrodo *E.coli* conjugate injections. Together, these data indicate that *H. bacteriophora* secretes products that modulate the *Drosophila* immune response and that these secretions may serve to promote the efficacy of *H. bacteriophora*'s symbiotic gram-negative bacteria and fellow entomopathogen, *Photorhabdus luminescens*.

# EXAMINING THE EFFECTS OF SUBLETHAL COPPER ON RAINBOW TROUT GILL CELLS EXPOSED TO VIRUS INFECTION

Samantha Kliewer[1], Scott Smith[1], Stephanie J. DeWitte − Orr[2]

[1]Wilfrid Laurier University, Department of Chemistry & Biochemistry, Waterloo, Ontario N2L 3C5, Canada
[2]Wilfrid Laurier University, Department of Biology, Waterloo, Ontario N2L 3C5, Canada

Copper is a potentially toxic element in aquatic environments, but it is also an essential nutrient that aids in the growth and development of various aquaculture species. Due to its endemic presence in Canadian waters, rainbow trout are likely exposed to copper during viral infections. Thus, it is important to understand what role copper plays in the antiviral process in these animals. Therefore, in this study, copper (II) sulfate will be used to examine whether or not exposure to metals makes rainbow trout gill cells in culture more susceptible to virus infection. Cell viability curves and $EC_{50}{}'s$ have been generated to determine what concentrations of copper (II) sulfate induces cell toxicity using two fluorescence indicate dyes, Alamar Blue and CFDA-AM. Viral susceptibility will be measured by first treating the cells with non-toxic concentrations of copper (II) sulfate for 24h, after which cells will be infected with viral hemorrhagic septicaemia virus (VHSV)-IVb. Control cultures will be infected with VHSV-IVb without copper (II) sulfate. Changes in resulting virus titres will be measured by $TCID_{50}$/mL on EPC cells. These results will determine whether or not copper exposure increases susceptibility to virus infection for rainbow trout gill cells in culture.

NACI Abstract

# Increasing efficacy of dsRNA against human pathogenic viruses using a nanocarrier

Aaron Lewis[1], Andrea Monjo[2], JD de Jong[2,3], Stephanie DeWitte-Orr[1,2]

Departments of Biology[1] and Health Sciences[2], Wilfrid Laurier University; Glysantis[3], Guelph ON

For decades the existence of broad-spectrum antibiotics has changed how bacterial infections have been treated and has saved countless lives. Viral infections are responsible for countless deaths each year, however, there are currently no broad-spectrum anti-viral drugs on the market that provide protection in the same way antibiotics do. The purpose of this project was to validate a novel nanoparticle's potential to enhance an immune stimulant's capability of eliciting a broad-spectrum antiviral response. The immune stimulant is double-stranded (ds)RNA, a replicative by-product of viral replication and potent inducer of a type I interferon response, in the form of the commercially available dsRNA, polyinosinic-polycytidylic acid (Poly IC). The nanoparticle (NP) is a proprietary molecule that is non-toxic and taken up by the cell. The two mammalian cell lines tested were Madin-Darby canine kidney (MDCK) cells and a human lung fibroblast cell line (MRC-5). Cells were infected with vesicular stomatitis virus with GFP inserted into its genome ($VSV_{GFP}$), this allowed use of fluorescence microscopy to measure viral infection. In both cell lines, Poly I:C+NP induced a stronger antiviral response compared with poly I:C alone The results show potential for this novel nanoparticle to increase the antiviral response induced by Poly I:C. Further research is required to test the Poly I:C+NP conjugate's efficacy against other viruses.

# ASSOCIATION OF FIBRINOGEN-RELATED PROTEIN 3 AND THIOESTER-CONTAINING PROTEIN FACILITATES RECOGNITION AND KILLING OF *SCHISTOSOMA MANSONI* IN THE SNAIL HOST

Hongyu Li[1,2], Jacob R. Hambrook[2], Emmanuel Pila[2], Jing Fang[1,2], Xinzhong Wu[1,3*], Patrick C. Hanington[2*]

[1] Ocean College, Qinzhou University, Qinzhou, Guangxi 535011, China;

[2] School of Public Health, University of Alberta, Edmonton, AB T6G2G7, Canada;

[3]College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, China;

* Patrick C. Hanington and Xinzhong Wu are corresponding authors.

The freshwater snail *Biomphalaria glabrata*, which serves as the natural intermediate host for *Schistosoma mansoni*, produces a secreted lectin, fibrinogen-related protein 3 (FREP3), which is known to be a central part of the anti-schistosome immune response. Expression of *Bg*FREP3 is increased following challenge of a resistant snail with *S. mansoni,* and knockdown of FREP3 by RNAi can alter the resistance phenotype. Evidence suggests that *Bg*FREP3 co-precipitates with secretion/excretion products and interacts with surface molecules of *S. mansoni* sporocysts. However, the underlying mechanism of *Bg*FREP3 function; how it binds to *S. mansoni* sporocyst surfaces, and then how recognition is translated into haemocyte engagement, activation, and ultimately parasite encapsulation, is still unknown. To better understand how *Bg*FREP3 functions, we first produced a recombinant *Bg*FREP3 (r*Bg*FREP3). In this study we demonstrate that r*Bg*FREP3 directly binds to *S. mansoni* sporocyst without any other soluble plasma immune factors. However, while sporocyst recognition can occur unaided, r*Bg*FREP3 still interacts with *B. glabrata* Thioester-containing Protein (*Bg*TEP) to form an immune complex at the sporocyst surface. In further functional validation experiments, our results show that the *Bg*FREP3-TEP immune complex significantly improves the ability of haemocytes from *S. mansoni*-susceptible *B. glabrata* snails (M-line) to kill sporocysts of *S. mansoni*.

# A SELECTION PLATFORM FOR ANTIBODY AFFINITY MATURATION IN THE MELANOMACROPHAGE CLUSTERS OF ZEBRAFISH.

Aradana Muthupandian, Doaa Waly, Harrison Anzinger, Tera Burris, Brad Magor.
Dept. Biological Sciences, University of Alberta, Edmonton, AB.

Germinal centres, the histologically distinct sites of antibody affinity maturation, have long been thought to be an evolutionary invention of the homeotherms. Indeed the question of if, or the degree to which, poikilothermic vertebrates affinity mature their antibody repertoires has remained contentious. We have previously demonstrated that the mediator of antibody (Ab) affinity maturation, Aicda, is expressed in cells within catfish melanomacrophage clusters (MMCs). We recently reported that we could isolate these clusters from spleen and kidney of zebrafish, to allow for analyses of Ab VDJ repertoires using BRILIA software. The conclusion of these analyses was that 1 - 3 B-cells nucleate a MMC and clonally expand while accumulating somatic mutations in their VDJ exons. This is akin to antibody affinity modification in germinal centres. These observations have since been verified using Alakazam clonal lineage reconstruction. To assess whether there is a mechanism for selection of these Ab affinity modified B-cells we vaccinated and then boosted goldfish and zebrafish with Alexa 647 conjugated BSA or KLH. Subsequent FACS analysis revealed that all Alexa 647+ spleen and kidney leukocytes also had the autofluorescence profiles of melanomacrophages. Confocal imaging revealed that the 'trapped' antigen was on the melanomacrophage cell surface. Furthermore magnetic beads coated with monoclonal anti-BSA could capture melanomacrophages from BSA vaccinated fish only, indicating that the Ag was retained intact on the cell surface. Collectively these results support the argument that MMCs are proto-germinal centres in the spleen and kidney of fishes.
*Funded through NSERC Canada.*

Oral presentation preferred

# CRISPR EDITING OF CHICKEN DF-1 CELL LINE TO EXPRESS DUCK RIG-I

Eric R. Fiesen[1,3], Yanna Xiao[2,3] and Katharine E. Magor[1,3]

*Department of Biological Sciences[1] and Medical Microbiology and Immunology[2] and Li Ka Shing Institute of Virology[3], University of Alberta, Edmonton, Alberta, Canada*

Chickens lack the detector for influenza, RIG-I, thus cannot generate a good response to influenza in epithelial cells, the first cells infected. As a result, the virus can replicate to a high titre and chickens are often the source of highly pathogenic influenza strains that can cause economic losses to poultry and pose health risks to humans. Ducks have RIG-I and initiate robust innate responses to influenza viruses. We believe that replacing RIG-I in chickens will augment their resistance to influenza virus. However, we do not know whether the duck RIG-I gene can be expressed and correctly regulated in chicken cells. Therefore, we characterized the duck RIG-I promoter and showed it is both constitutively expressed and interferon-inducible in chicken cells. To modify chicken cells, we designed a repair plasmid that can be used to knock-in duck RIG-I with homology arms for targeting the gene to the location on the Z chromosome from which the gene was lost in galliform birds. We also modified a CRISPR/Cas9 plasmid for expression of small RNAs in chicken cells, and included the guide RNA sequences for precise targeting of double-strand breaks. To test our constructs for CRISPR-mediated gene knock-in we have modified the embryonic fibroblast DF-1 cell line to express duck RIG-I under the control of its own promoter. Testing of these cells for correct insertion, fidelity of RIG-I expression and influenza resistance is underway. These experiments provide proof-of-principle that CRISPR can be used for modification of chicken primordial germ cells for generation of transgenic birds.

**PRE-TREATMENT WITH POLY(I:C) CONFERS PARTIAL PROTECTION AGAINST FROG VIRUS 3 IN TWO PERMISSIVE XENOPUS LAEVIS SKIN EPITHELIAL CELL LINES**

Maxwell P. Bui-Marinos, Marie-Claire D. Wasson, Barbara A. Katzenback

*Department of Biology, University of Waterloo, Waterloo, Ontario, Canada*

Frog Virus 3 (FV3) is known to cause mass mortalities in amphibian populations on a global scale. While skin acts as the first barrier of defense between a host and its environment, the role that frog skin epithelial cells play in recognition and response to FV3 is largely unknown. To better elucidate skin epithelial cell-FV3 interactions, we used newly established epithelial cell lines from *Xenopus laevis* dorsal skin (Xela DS2) and ventral skin (Xela VS2) tissues. To determine susceptibility to FV3, Xela DS2 and Xela VS2 were incubated with a multiplicity of infection (MOI) ranging from 0.002 to 20 and cytopathic effects and viral titre monitored. Dose- and time-dependent increases in infective virions, coinciding with cytopathic effects and loss of cell adherence, were observed. Assessment of Xela DS2 and Xela VS2 immunocompetence and response to FV3 were performed by detection of pro-inflammatory and anti-viral transcripts via RT-qPCR following treatment with poly(I:C), FV3 or UV-inactivated FV3. While both cell lines exhibited an increase in proinflammatory and antiviral transcripts following poly(I:C) stimulation, no change in transcript levels were observed following challenge with FV3 or UV-inactivated FV3. However, poly(I:C) pre-treatment of Xela DS2 and Xela VS2 prior to FV3 challenge reduced cytopathic effects and production of infective virions. These data demonstrate that frog skin epithelial cells are permissive to FV3 and, while FV3 appears to effectively supress key innate immune functions in Xela DS2 and Xela VS2, pre-treatment of these cells with poly(I:C) can effectively limit viral replication.

**Protein expression and characterization of antibodies to IgT$^+$-B cell-specific perforins**

Jack Martin1, Yasuhiro Shibasaki[2], J. Oriol Sunyer[2]

*1 Department of Chemistry, Brown University, Providence, RI.*
*2 Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, 19104.*

Perforin is a protein known to have cytolytic and membrane disruptive activity. It is well known to be expressed in cytotoxic T cells and NK cells. However, through transcriptomic analysis, we have surprisingly found that rainbow trout IgT$^+$ B cells expresses several isoforms of perforin. We have confirmed high expression levels of these perforin isoforms, including prf1-like-B, prf1-like-C and prf1-like-D in IgT$^+$ B cells by RT-PCR. To understand further the role of these perforin molecules at the protein level, and their distribution in fish leukocytes, we developed polyclonal and monoclonal antibodies against all perforin isoforms. To this end, we constructed plasmids that express perforin1-like-B and -D isoforms, using HEK cell lines. Interestingly upon transfection, HEK cells showed a significant degree of mortality, which was possibility related to the cytotoxic capacity of the perforin proteins. However, the cell lines produced enough perforin proteins enable to the immunization of guinea pigs and mice for the development of polyclonal and monoclonal antibodies. Specificity of the antibodies was evaluated by western blotting on purified recombinant perforin isoforms. Finally, we developed immunofluorescence protocols that enabled the identification of the several perforin isoforms on trout lymphoid tissues. Interestingly we found a significant percentage of IgT$^+$ B cells on these tissues that stained positively for the various anti-perforin antibodies, whereas the staining on IgM$^+$ B cells was negligible. We are currently analyzing the potential expression of these perforins on different trout leukocyte populations with the use of recently developed antibody reagents against several trout lymphocytes, including gamma/delta and Natural Killer cells. In conclusion our protein expression analysis appears to confirm the cytolytic capacity of all analyzed trout perforins. More importantly, the development of perforin-speficic antibody reagents allowed us to confirm the protein expression of several perforin isoforms by IgT$^+$ B cells. These antibodies will be critical to further the structural and functional characterization of trout perforins and their roles in IgT$^+$ B cells.

# CHARACTERIZATION OF ORGANIZED SITES OF B CELL SELECTION IN THE NURSE SHARK SPLEEN

Hanover Matz[1,2] and Helen Dooley[1,2]

[1]Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD 21201, USA. [2]Institute of Marine and Environmental Technology (IMET), Baltimore, MD 21231, USA

B cell clone selection and antibody affinity maturation are fundamental mechanisms of the adaptive immune response. Historically affinity maturation was believed to occur only in endothermic vertebrates, with selection taking place in germinal centers formed in the spleen and lymph nodes. However, it has since been proven that cold-blooded vertebrates are also capable of some level of affinity maturation despite lacking germinal centers and true follicular dendritic cells. This finding led us to hypothesize that a primordial B cell selection structure preceded the complex germinal centers present in mammals. Further, these structures may be retained in cartilaginous fishes, such as sharks, the oldest extant taxonomic group that possess adaptive immunity based on immunoglobulins.

Through immunization studies utilizing the fluorescent antigen phycoerythrin (PE), we have identified organized sites of B cell selection in an ectothermic vertebrate, the nurse shark (*Ginglymostoma cirratum*). Using immunofluorescent microscopy and RNA fluorescent in situ hybridization experiments we have located, and begun to characterize, the cellular architecture of the B cell selection sites in nurse shark spleen. Our results show distinct sites where PE is presented by unknown antigen presenting cells to B cell clones expressing IgNAR (immunoglobulin new antigen receptor), a heavy-chain only immunoglobulin class and major contributor to the shark humoral adaptive response. We hypothesize that these sites facilitate selection of B cell clones undergoing somatic hypermutation, helping increase the binding affinity of the immune response. Future studies will continue to characterize the cellular and molecular underpinnings that facilitated the evolution of affinity maturation.

Authors: Connor C. McGuire[1], Jacques Robert[1, 2]

Affiliations:[1]Department of Environmental Medicine, [2]Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester NY.

# Exposure to a mixture of suspected thyroid disrupting chemicals alters intrathymic T cell differentiation in *Xenopus laevis* tadpoles

## Abstract

Thyroid hormones (TH) control postembryonic vertebrate development through thyroid receptor (TR) signaling. Deficiencies in TH production during development are known to contribute to immunodeficiency in humans and poorer outcomes in autoimmune disease models. To investigate whether endocrine disrupting chemicals that act on the TH pathway can alter the development of the immune system, we have tested a mixture of chemicals used in unconventional oil and gas extraction that are suspected to be TR antagonists or TH synthesis inhibitors. We verified TR antagonist activity by exposing pre-metamorphic *Xenopus laevis* tadpoles to 1.25 nM of the TR agonist triido-L-thryonine (T3) and T3 combined with 1 µg/L or 10 µg/L of the mixture, and discovered that the 10 µg/L dose of the mixture prevented T3's induction of the *klf9* expression in the thymus, a key TR-responsive gene also critical for T cell development. To determine the effect of this mixture on intrathymic T cell development at steady states, we exposed *Xenopus laevis* tadpoles to 1 µg/L or 10 µg/L of the mixture or DMSO control for two weeks and evaluated changes in thymocyte subsets using two-color flow cytometry. Exposure to the 10 µg/L dose of the mixture increased the number of CD8+, CD4+, double positive, and double negative thymocytes, while reducing a population of less well characterized CD8+/CD5- cells. Thus, our data suggest that disruption to the TH axis during tadpole development induces acute alterations to the thymus perturbating T cell differentiation.

# THE THIRD DOMAIN OF T CELLS

Robert D. Miller

*Center for Evolutionary and Theoretical Immunology, Department of Biology, University of New Mexico, Albuquerque, NM*

Conventional T cells are characterized as being either αβ T cells or γδ T cells based on the composition of their T cell receptor (TCR). All jawed-vertebrates, with the possible exception of squamate reptiles, have both αβ and γδ T cells. In addition to αβ and γδ T cells, the non-eutherian mammals (marsupials and monotremes) have a third lineage, the γμ T cell. *TCRμ* has a distinct genomic organization and encodes a third immunoglobulin domain in the extracellular chain. To investigate the function of γμ T cells, single cell RNA sequencing was performed on opossum splenocytes. γμ T cells make up approximately 10% of splenocytes in the opossum, are the second most common T cell in the spleen after αβ T cells, and appear to be absent from peripheral blood. γμ T cells have a distinct transcriptome, relative to αβ T cells. Transcriptome analyses reveal that a majority of γμ T cells are $CD8^+$ but express CD8 in the CD8αα form. None are $CD4^+$. In addition, γμ T cells express an unusual combination of killer associated markers and, curiously, olfactory receptors. Globally, the transcriptomes of γμ T cells are highly distinct from that of αβ T cells. Opossum γδ T cells however are a split group, sharing features of γμ T cells and some αβ T cells. In summary, γμ T cells are an ancient T cell type in mammals, that was lost in the eutherians (*e.g.* humans), whose function is unknown but appears distinct from conventional T cells.

# MONITORING FROG VIRUS 3 (FV3) IN DUNDAS VALLEY *AMBYSTOMA JEFFERSONIANUM* POPULATIONS

Emma Monod[1], Dr. Levi Moore[1], Dr. Stephanie Dewitte-Orr[1,2]

[1] *Department of Biology, Wilfrid Laurier University*

[2] *Department of Health Sciences, Wilfrid Laurier University*

The Jefferson salamander, *Ambystoma jeffersonianum*, has been classified as an endangered species in Ontario after suffering from a 90% population decline over the past 33 years. Species conservation efforts are focused on preventing habitat loss primarily by mitigating the degradation of key woodland and breeding pond habitats. A significant cause of population decline in the population of *A. jeffersonianum* is the low survival rates (0-0.7%) of pre-metamorphosis larvae. Infection by the Ranavirus that commonly affects amphibian populations, Frog Virus 3 (FV3), may be threatening pre-metamorphosis larvae as well as the adult population. FV3 causes organ necrosis and hemorrhaging and has been the cause of significant amphibian population die-offs. The Jefferson salamander meets the criteria for amphibian species that have a high susceptibility to the virus; they have a short larval phase, have restricted distributions and inhabit semi-permanent breeding sites. Furthermore, agricultural threats to salamander habitats have also increased the likelihood for infection by FV3, which may be attributed to the leeching of agricultural runoff into breeding ponds. FV3 has been confirmed in wild populations of *A. jeffersonianum* in Wisconsin USA, however its presence has not yet been confirmed in Canada despite observed declines in the Canadian population consistent with the presence of FV3. As such, DNA was collected from adult *A. jeffersonianum* skin swabs, from animals captured in the Dundas valley region in order to test for the presence of FV3 within this population.

**The development of a protein level assay for detection and quantification of tumor necrosis factor alpha in rainbow trout (*Oncorhynchus mykiss*)**

**Alisha Moynahan, Aaron Frenette, Tania Rodríguez-Ramos and Brian Dixon**

Department of Biology, University of Waterloo, Waterloo, ON

Over the next few decades, climate change will cause fluctuations in the abiotic environment (water temperature and oxygen levels) which will leave farmed salmonids, including rainbow trout (*Oncorhynchus mykiss*), more susceptible to infectious disease agents. Cytokines, such as tumor necrosis factor alpha (TNFα), regulate immune function in response infections/stress and represent key functional targets for assessing fish health and immunocompetency. Thus, the development of immunological tools (polyclonal antibodies) that enable the detection and quantification of TNFα in cell culture and primary cells was the goal of this project. A rainbow trout monocyte/macrophage cell line was stimulated with zymosan (yeast glucan) to stimulate production of native TNFα. Protein profiles, analyzed by Western blot, from cells and conditioned media demonstrated the presence of membrane-bound and soluble TNFα, respectively. Furthermore, Western blot analysis of rainbow trout splenocytes, stimulated with heat-killed *Vibrio anguillarum*, detected soluble TNFα in the conditioned media. These results support the use of these antibodies in the generation of a quantitative enzyme-linked immunosorbent assay capable of determining the level of TNFα in fish serum. These assays will contribute to a better functional understanding of fish cytokines and provide an improved method for assessing the health status of cultured fish populations, thereby enabling selection of more efficacious pathogen-directed vaccines/treatments. The development of protein level cytokine assays will ultimately help sustain the salmonid aquaculture industry, which generates over a billion dollars in economic activity in Canada annually, as it adapts in the face of a changing aquatic environment.

**N= 245 words**

# CHARACTERIZING CYTOKINE AND miRNA RESPONSES IN VARIABLE STRESS-RESPONDING SHEEP

Danielle Naylor[1], Ankita Sharma[1], Ziwei Li[1], Angela Canovas[1], Bonnie Mallard[2], Niel Karrow[1]

[1]*Department of Animal Biosciences, University of Guelph, Guelph, ON*
[2]*Department of Pathobiology, University of Guelph, Guelph, ON*

Genetic selection of livestock for enhanced stress resilience may be a strategy to mitigate microbial stressors and limit use of antimicrobials. The present study identified high (HSR, >300 nmol/L, n=5), middle (MSR, 127-208 nmol/L, n=5) and low (LSR, <100 nmol/L, n=5) stress responding Rideau-Arcott sheep based on their peak cortisol response to systemic bacterial lipopolysaccharide (LPS) endotoxin challenge, which is moderately heritable (h2≅0.3). Rectal temperature and a panel of serum cytokines and immune-related microRNAs were measured before and 2-6 hr post-LPS challenge to characterize immune function in these variable stress-responding sheep. Significant differences were observed in rectal temperatures among the stress-responding groups at 4 hr, with HSR sheep having the strongest fever response. Pro-inflammatory (IL-6, IFN-γ, IP-10, TNF-α, chemokine CCL3) and anti-inflammatory cytokines (IL-10) were significantly highest in HSR sheep post-LPS challenge. The expression of miR-145, which targets the TLR-4 pathway, was significantly higher in LSR sheep post-LPS challenge compared to HSR sheep. Conversley, miR-223, known to regulate granulopoiesis, did not demonstrate significant differences in expression among the groups, although there was a significant response over time ($p<0.05$). These results demonstrate that serum cytokines and miRNAs are differentially expressed in variable stress responding sheep, which suggests that immune function may be affected in sheep by selection of stress resilience.

# THE *HYDRACTINIA* ALLORECOGNITION COMPLEX ENCODES A LARGE FAMILY OF NOVEL IMMUNOGLOBULIN SUPERFAMILY MEMBERS

Aidan Huene[1,2], Steven Sanders[1,2], Ruoxu Chen[3], Nicole Shigiltchoff[4], Zhiwei Ma[1,2], Anh-Dao Nguyen[5], Sergey Koren[5], James C. Mullikin[6], Adam Phillippy[5], Christine Schnitzler[5,7,8], Andreas Baxevanis[5], and Matthew L. Nicotra[1,2,9]

[1]Starzl Transplantation Institute, Department of Surgery, University of Pittsburgh, Pittsburgh, PA, [2]Center for Evolutionary Biology and Medicine, University of Pittsburgh, Pittsburgh, PA [3]School of Medicine, Tsinghua University, Beijing, China, [4]UPMC Hillman Cancer Center Academy, Hillman Cancer Center, Pittsburgh, PA, [5]Computational and Statistical Genomics Branch, Division of Intramural Research, National Human Genome Research, National Institutes of Health, Bethesda, MD, [6]NIH Intramural Sequencing Center, National Human Genome Research Institute, National Institutes of Health, Rockville, MD, [7]Whitney Laboratory for Marine Biosciences, University of Florida, St. Augustine, FL, [8]Department of Biology, University of Florida, Gainesville, FL, [9]Department of Immunology, University of Pittsburgh, Pittsburgh, PA

Colonial marine invertebrates are capable of allorecognition—the ability to discriminate between their own tissues and those of conspecifics. In *Hydractinia symbiolongicarpus*, a cnidarian model of allorecognition, compatible colonies fuse permanently, while incompatible colonies fuse temporarily or reject aggressively. These responses are controlled by a genomic region called the allorecognition complex (ARC). Previously, we and others identified two ARC-encoded allorecognition genes, *Allorecognition 1* (*Alr1*) and *Allorecognition 2* (*Alr2*). Both are single-pass transmembrane proteins with highly polymorphic extracellular regions. Allelic isoforms of Alr1 and Alr2 engage in homophilic binding only with isoforms of nearly identical sequence, suggesting a central role in self/non-self discrimination. Partial sequencing of the ARC revealed additional *Alr*-like sequences, but the full extent of this gene family was heretofore unknown.

Here, we report a nearly complete 11.5 Mb reference sequence for the ARC. Annotation of this sequence reveals a family of 28 *Alr* genes and 15 *Alr* pseudogenes. Nearly half of *Alr* genes reside in one of three clusters, and mapping data is consistent with at least one previously unknown allodeterminant. Alr proteins possess extracellular regions consisting of tandem domains predicted to adopt immunoglobulin-like and fibronectin type III like folds. Like Alr1/2, several newly discovered Alr proteins are also capable of homophilic binding across opposing cell membranes. Comparative analysis across several ARC haplotypes reveals high levels of allelic polymorphism, gene duplication, and copy number variation, suggesting common mechanisms of genomic evolution with mammalian KIR and Ly49 systems.

# THE MULTIDIMENSIONAL CHARACTERIZATION OF AID AND AID-LIKE ENZYMES IN EARLY-EVOLVED SPECIES

Emma Quinlan[1], Chris Amemiya[2], Ellen Hsu[3], Stephen Holland[4], Lesley Berghuis[1], Justin King[1], Thomas Boehm[4], and Mani Larijani[1]

*[1]Department of Biomedical Sciences, Faculty of Medicine, Memorial University of NL, Canada; [2]Department of Biology, University of Washington, WA, United States; [3]Department of Physiology and Pharmacology, SUNY Downstate Medical Center, NY, United States; [4]Max Planck Institute of Immunobiology and Epigenetics, 79108 Freiburg, Germany*

The immunoglobulin (Ig)-based adaptive immune system (AIS) evolved ~500 mya in jawed vertebrates, while the jawless vertebrates, although lacking many AIS components such as Ig, exhibit their own variable lymphocyte receptor (VLR)-based AIS. An evolutionarily-conserved hallmark of the Ig-based AIS is diversification of Ig-class antibodies through Somatic Hypermutation (SHM) and/or Class Switch Recombination (CSR) of Ig genes, which are initiated by the DNA-mutating enzyme Activation-induced cytidine deaminase (AID). The VLR-based AIS is thought to also involve activities of AID-like enzymes, the mechanisms of which remain unknown. Interestingly, while each clade of jawed vertebrate fishes possesses genetically-diverse AID, the jawless vertebrate lamprey has *multiple* AID-like genes, with the unique phenomenon of varying expression patterns between individuals of the same species. To understand the role of AID in AIS evolution, and to elucidate structure-function relationships in these distinct AID proteins, we structurally and biochemically examined AID from the sea and freshwater lampreys, nurse shark, zebrafish, tetraodon, coelacanth, and human. We found the defining properties of human AID were conserved across all jawed vertebrates, while other characteristics, like optimal temperature, diverged. Furthermore, the lamprey AID orthologues exhibited varying pH sensitivities and enzyme activity levels, similar to human AID and its APOBEC relatives, suggesting unique roles for these AID orthologues in the lamprey AIS. This is the first study to biochemically and structurally characterize this number and diversity of AID and AID/APOBEC-like enzymes. This multidimensional approach of multi-species *in silico* structural analysis and biochemical characterization exemplifies a comprehensive method for understanding enzyme evolution.

*This research is supported by funds from the Canadian Institute of Health Research (CIHR), Natural Sciences and Engineering Research Council of Canada (NSERC) and Memorial University of Newfoundland.*

# EXPERIMENTAL PERTURBATION OF THE SEA LAMPREY VARIABLE LYMPHOCYTE SYSTEM USING CRISPR/CAS9

Jonathan P. Rast[1]

[1]Department of Pathology and Laboratory Medicine, Emory University.

Lampreys and hagfish comprise about 60 species of jawless vertebrates that collectively represent an anciently derived sister group to the jawed vertebrates. While, the jawed vertebrates use diversified immunoglobulin (Ig) and T cell receptors (TCR) as the basis of their adaptive immune system, the jawless vertebrates instead use somatically diversified leucine rich repeat proteins, the variable lymphocyte receptors (VLRs). Well-formulated comparisons between these systems potentially provide a long-sought window into the deep origins of vertebrate adaptive immunity. Structurally, the VLR proteins are completely unrelated to Ig/TCR, yet they have analogous functional characteristics and are rooted in a cellular system with distinct similarities to jawed vertebrate B and T cells. Since their discovery 15 years ago, much has been learned about the structure, diversity and expression of the VLRs and their response to immune challenge. Still, fundamental questions remain about how the system functions and how it relates to the jawed vertebrate adaptive system. Many of these lines investigation require new experimental strategies to manipulate the system *in vivo*. As one approach, we have begun CRISPR/Cas9 experiments to probe causal linkage among VLRs and their predicted assembly and regulator factors in the sea lamprey. We are also interested development of the VLR system in the early feeding larva. I will discuss our findings to date and plans for future research.

**DEVELOPMENT AND OPTIMIZATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) TO QUANTIFY IL-1β IN SALMONIDS**

Tania Rodríguez-Ramos[1], Aaron Frenette[1], Shawna L. Semple[1], Fabio Zanuzzo[2], Huming Guo[1], Cheryl Soulliere[1], George Heath[1], Jack Iwanczyk[3], A. Kurt Gamperl[2] and Brian Dixon[1]

[1]University of Waterloo, 200 University Avenue West, Waterloo, ON. N2L 3G1.
[2] Department of Ocean Sciences, Memorial University, St. John's, NL. A1C 5S7.
[3]Cedarlane Laboratories Limited, 4410 Paletta Court, Burlington, ON. L7L 5R2.

Cytokines are small signaling proteins that regulate host immune responses during bacterial and viral infection. In teleosts, genes encoding several cytokines have been identified and transcriptomic studies support the theory that fish cytokines have similar biological function to mammalian cytokines. However, the degree to which cytokine expression profiles predict protein abundance remains unknown due to the absence of quantitative assays for these proteins. Here, we describe the development and optimization of a sandwich Enzyme-Linked Immunosorbent Assay (ELISA) capable of quantifying <10 pg $mL^{-1}$ of interleukin-1 beta (IL-1β) in fish cells and tissues. The optimization process of this ELISA involved the selection and adjustment of antibodies, enzymatic reporters and buffers to obtain a robust immunoassay with high sensitivity, specificity and reproducibility. The use of a commercial diluent was necessary to equalize the sample and standard matrices, and to inhibit sample complement activity, while polyethylene glycol (PEG) addition allowed for a lower detection limit for IL-1β in the samples. IL-1β was successfully measured in a trout (*Oncorhynchus mykiss*) monocyte/macrophage-like cell line (RTS11), trout splenocytes, and in plasma from trout and Atlantic salmon (*Salmo salar*) exposed to killed pathogen or supra-optimal temperatures. The development of reliable quantitative immunoassays will greatly improve our understanding of fish immunology. Further, it will support management practices and could lead to therapeutics in fish aquaculture, both of which could save the industry millions of dollars.

**IMMUNE DEFENSES OF AMPHIBIANS AGAINST BATRACHOCHYTRIUM FUNGI**

Louise A. Rollins-Smith[1], Laura K. Reinert[1], Mitchell Le Sage[1], Thomas Umile[2], Kevin P.C. Minbiole[2], E. Davis Carter[3], Debra L. Miller[3], and Matthew J. Gray[3]

[1]*Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN*
[2]*Department of Chemistry, Villanova University, Villanova, PA*
[3] *University of Tennessee Institute of Agriculture, Knoxville, TN*

Amphibians have been declining around the world for more than four decades. Contributing to these declines are the chytrid fungi, *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*), which cause the disease chytridiomycosis. Amphibians have complex immune defenses against fungi, but *Batrachochytrium* fungi have a number of counter-defenses. Previously, we identified three small metabolites that inhibit lymphocyte proliferation. We hypothesize that the fungi respond to stress by production and release of these mediators to evade immune destruction. *Bsal* is the newest amphibian pathogen that causes death as it enters new populations, and it appears to produce similar inhibitory metabolites. Here, we present preliminary evidence that innate skin secretions of Eastern newts, *Notophthalmus viridescens,* contain a mixture of proteins that reduce the viability of infectious *Bsal* zoospores. The proteins appear to be more abundant when induced from animals held in cold conditions (6 °C) in comparison with warmer newts (at 14 °C or 22 °C). In *Bsal* exposure trials, pathogen loads were also reduced at 6 °C in comparison with animals exposed at 14 °C or 22 °C. Although cold temperature did not impair production of the defensive proteins, they were only partially protective for *Bsal*-exposed newts. Newts at 6 °C developed chytridiomycosis and died more slowly than at 14 °C; however, mortality was similar between temperatures. Thus, it appears that temperature may plan a critical role in innate skin defenses against *Bsal* chytridiomycosis, and those defenses alone are insufficient to prevent development of chytridiomycosis in this susceptible host species.
Funding NSF IOS-1557634, IOS-155759, DEB-1814520.

# Conserved mechanisms underlying loss of tolerance to allogeneic tissues in *Botryllus schlosseri* chimeras

Benyamin Rosental[1,2*], Mark Kowarsky[3*], Jun Seita[2,4], Daniel M. Corey[2], Katherine J. Ishizuka[2], Karla J. Palmeri[2], Shih-Yu Chen[5], Rahul Sinha[2], Jennifer Okamoto[6], Gary Mantalas[7], Lucia Manni[8], Tal Raveh[2], D. Nathaniel Clarke[2], Aaron M. Newman[2], Norma F. Neff[6], Garry P. Nolan[5], Stephen R. Quake[6,7,&], Irving L. Weissman[2,6&] and Ayelet Voskoboynik[2,6&].

[1] French Associates Institute for Agriculture and Biotechnology of Drylands, the Jacob Blaustein Institutes for Desert Research, Ben Gurion University of the Negev, Israel. [2]Institute for Stem Cell Biology and Regenerative Medicine, Ludwig Center, and Hopkins Marine Station, Stanford University, CA, USA. [3]Department of Physics, Stanford University, CA, USA. [4]AI based Healthcare and Medical Data Analysis Standardization Unit, Medical Sciences Innovation Hub Program, RIKEN, Japan. [5]Department of Microbiology and Immunology, Stanford University School of Medicine, USA. [6]Chan Zuckerberg Biohub, San Francisco CA, USA. [7]Departments of Applied Physics and Bioengineering, Stanford University, CA, USA [8]Dipartimento di Biologia, Università degli Studi di Padova, Italy. *,& Equal contribution.

The mechanisms that sustain immunological non-reactivity are the basis for understanding the maintenance of tissue in syngeneic and allogeneic settings. While most transplantation rejection occurs due to the adaptive immune response, the pro-inflammatory response of innate immunity is necessary for the activation of adaptive immunity - both in syngeneic and allogeneic settings. We study a unique chordate model, *Botryllus schlosseri*, that lacks a classic adaptive immune system, yet has the ability to reject allogeneic individuals or form chimeras with compatible animals. This organism demonstrates three major innate immunity responses: non-inflammatory program cell removal, acute rejection (between non-compatible animals) and allogeneic resorption (between compatible colonies that formed chimeras). Using flow cytometry, whole-transcriptome sequencing of defined cell populations and tissues, and diverse functional assays, we isolated 24 endpoint *B. schlosseri* cell populations, identified hematopoietic stem cell (HSC), progenitors, immune-effector cells, and the HSC niche. Furthermore, we identified a *B. schlosseri* cytotoxic cell population originating from large granular lymphocyte-like cells and demonstrated their function in acute and chronic rejection processes. Studying the molecular and cellular framework underlying loss of tolerance to allogeneic tissues within the *B. schlosseri* chimera, we found that developmental cell death programs license cytotoxic cells to eliminate histocompatible partners. This study demonstrates that interactions between pro-inflammatory and damaged tissue removal, lead to robust cytotoxic and phagocytic clearance programs within the allogeneic microenvironment.

References: Corey DM*, Rosental B*, Kowarsky M, Sinha R, Ishizuka KJ, Palmeri KJ, Quake SR, Voskoboynik A, Weissman IL. Developmental cell death programs license cytotoxic cells to eliminate histocompatible partners. Proc Natl Acad Sci U S A, 2016.

Rosental B, Kowarsky M, Seita J, Corey DM, Ishizuka KJ, Palmeri KJ, Chen SY, Sinha R, Okamoto J, Mantalas G, Manni L, Raveh T, Clarke DN, Tsai JM, Newman AM, Neff NF, Nolan GP, Quake SR, Weissman IL, Voskoboynik A. Complex mammalian-like haematopoietic system found in a colonial chordate. Nature, 2018.

# MOLECULAR DRIVERS OF LYMPHOCYTIC ORGANIZATION AT VERTEBRATE MUCOSAL SITES

RYAN HEIMROTH, IRENE SALINAS

Department of Biology, University of New Mexico, Albuquerque

The aggregation and organization of lymphocytes at mucosal lymphoid tissues in structures such as Peyer's patches (PP) and tonsils is thought to be a unique feature of the mucosal immune system of endotherms. However, cold-blooded vertebrates also have lymphocyte aggregates at mucosal sites, albeit less organized. This progressive organization of the mucosal immune system has been attributed to the expansion and diversification of members of the tumor necrosis factor superfamily (TNFSF). This hypothesis is based on mammalian studies using different mouse knock-outs in which organized mucosa-associated lymphoid structures (O-MALT) or lymph nodes are missing. However, an unbiased search of molecular drivers of O-MALT formation across vertebrate groups has not been conducted thus far. Revisiting the TNFSF hypothesis indicated a lack of support for the progressive diversification of TNFSF from bony fish to mammals based on BLAST and HMM searches of all available genomes and transcriptomes. We next performed RNA-Seq from the interbranchial lymphoid tissue of rainbow trout, the nasal lymphoid aggregates of African lungfish, avian ceacal tonsils and mouse PP and LN. After subtracting genes expressed in non-O-MALT negative control tissues from each species, we identified unique and shared suites of genes in the different O-MALT structures. Specifically, we identified that genes with predicted neuroactive ligand-receptor functions are enriched in all vertebrate O-MALT transcriptomes and that lungfish and mouse O-MALT are enriched in olfactory receptors genes. Our results provide a new theoretical framework for understanding the molecules required for the formation and organization of lymphocytic structures at vertebrate mucosal tissues.

# INNATE-LIKE T CELLS IN TROUT NASAL IMMUNITY

Pankoj K. Das, Aurora Kraus, Ali Sepahi, Irene Salinas

Department of Biology, University of New Mexico
Email: isalinas@unm.edu

T lymphocytes are known for their vital role in adaptive immune responses of jawed vertebrates. However, non-conventional innate-like T cells also play critical immunological roles. We previously identified a population of CD8 T cells that rapidly infiltrates the olfactory organ (OO) of rainbow trout in response to nasal viral delivery. Given the rapid nature of this response we hypothesized that these T cells are non-specific. To test this hypothesis, rainbow trout received intranasally one of the following treatments: vehicle, live *Edwardsiella ictaluri*, killed *Yersinia ruckerii*, poly I:C, LPS, acetic acid (a noxious substance to fish OO) and DSS (breaks down epithelial barriers). Changes in the percentages of CD8+ T cells in the trout OO were evaluated 15 min later by flow cytometry. Both bacterial treatments but not poly I:C, LPS, acetic acid or DSS resulted in CD8 T cell infiltration into the trout OO. In order to know whether rapid innate-like T cell responses occur upon secondary pathogen encounter, we immunized trout intranasally with live attenuated IHNV or vehicle and 28 days later, the two groups either received vehicle or IHNV intranasally. Results show that rapid infiltration into the OO only occurs upon the first exposure, suggesting that innate-like T cells are not required during the secondary antiviral immune response. Combined, these data indicate that nasal innate-like T cell responses are not specific and can be elicited by multiple microbial stimuli but tissue damage is not sufficient to induce such responses.

**CHARACTERIZATION OF INNATE RESPONSES INDUCED BY DSRNA FORMULATED WITH CATIONIC NANOPARTICLES IN RAINBOW TROUT**

Kayla Samms[1], Tamiru Alkie[1], Jondavid de Jong[1,2], Kristof Jenik[1], Karl Klinger[2], Stephanie DeWitte-Orr[1]

[1]Department of Health Sciences, Wilfrid Laurier University**,** Waterloo, ON
[2]Mirexus Biotechnologies, Guelph, ON

The modulation of innate immunity by pathogen-associated molecular patterns (PAMPs), or synthetic mimics, that are recognized by host cell surface or intracellular receptors has profound effects on the generation of a desired innate immune response. Polyinosinic:polycytidylic acid (polyI:C) is a synthetic double stranded RNA (dsRNA) molecule known for inducing type I interferons (IFNs), interferon stimulated genes (ISGs), and an antiviral state in teleost species, following parenteral injection. We hypothesized that both local and systemic innate immune responses induced by poly(I:C) could be enhanced by oral delivery with a biodegradable, non-toxic nanoparticle (NP) in rainbow trout. Individually anesthetized rainbow trout received an oral gavage containing a meal of ground commercial trout pellets moistened with either water, the dsRNA-NP complex or a free dsRNA solution. Forty-eight hours after the first gavage, the fish were also boosted orally with the same formulations as indicated above. At 24 and 48hr post-primary gavage and at 24hr and 7 days post-boost, fish were euthanized and the expression of IFN1 and ISGs (vig-3, Mx1) were quantified in three portions of the intestine (proximal, middle and distal) and head kidney by qPCR. The results indicated that the dsRNA-NP complex induced a higher level of expression of IFN1 and ISGs in almost all segments of the intestine and head kidney. Additionally, in the intestine, none of the formulations induced histopathological lesions. The results of this study have significant implications for the aquaculture industry to exploit innate immunity as the primary defense mechanisms against viral pathogens.

# BIOINFORMATICS ANALYSES OF B-CELL REPERTOIRE EXPRESSION IN THE GRAY SHORT-TAILED OPOSSUM (*MONODELPHIS DOMESTICA*)

Andrea L. Schraven[1], Robert D. Miller[2], Hayley J. Stannard[3,4], Oselyne T.W. Ong[5], Victoria L. Hansen[2], Kimberly A. Morrissey[2], Julie M. Old[1]

[1]School of Science and Health, Western Sydney University, Penrith, New South Wales, Australia
[2]Center for Evolutionary and Theoretical Immunology, Department of Biology, University of New Mexico Albuquerque, New Mexico, USA
[3]School of Life and Environmental Sciences, and Charles Perkins Centre, The University of Sydney, Sydney, New South Wales, Australia.
[4]Charles Sturt University, School of Animal and Veterinary Sciences, Wagga Wagga, New South Wales, Australia
[5]QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia

B-cells are key to humoral immunity, are found in multiple lymphoid organs, and have the unique ability to mediate the production of antigen-specific antibodies in the presence of pathogens. Marsupial B-cell investigations have become increasingly important in understanding an adaptive immune system that develops primarily postnatally. In comparison to eutherians and monotremes, marsupial B-cells have four Immunoglobulin (Ig) heavy (H) chain isotypes (IgA, IgG, IGM and IgE), defined by their constant regions (Cα, Cγ, Cμ and Cε respectively), and two light (L) chain isotypes; lambda (λ) and kappa (κ). Preliminary bioinformatics of single cell RNA sequencing results indicated a high diversity of repertoire expression between the spleen and peripheral blood of the Gray short-tailed opossum (*Monodelphis domestica*). High expression levels of IgM were consistently observed in both tissues; IgG were expressed more so in the spleen whereas IgA had a higher expression in the peripheral blood. Analysis of the B-cell light chain revealed a Igλ to Igκ ratio of 2:1 in both tissues. Annotation of the gene segments show Igλ and Igκ have highly diverse variable (V) gene families suggesting a higher rate of complexity than the IgH loci and therefore has a greater contribution to the overall antibody repertoire.

**BACTERIAL COLONIZATION, INFECTION, AND IMMUNITY IN THE PURPLE SEA URCHIN LARVA**

Nicholas W. Schuh[1, 2], Andreas Heyland[2], Jonathan P. Rast[1, 3, 4]. [1]Department of Medical Biophysics, University of Toronto, Toronto, ON; [2]Department of Integrative Biology, University of Guelph, Guelph, ON; [3]Department of Immunology, University of Toronto, Toronto, ON; [4]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA.

Exposure to bacteria during development has wide-ranging effects on animal biology but can also inhibit growth or cause disease. The immune system is the prime mediator of these microbial interactions, and is itself shaped by them. Our group uses larvae of the purple sea urchin (*Strongylocentrotus purpuratus*) as an experimental model for colonization, immune development, and infection. Previously, we identified multiple larval immunocytes and characterized their functions during exposure to the model pathogen *Vibrio diazotrophicus*. Subsequently, we have shifted focus to commensal and/or opportunistic larva-associated bacteria isolated from larval samples. We visualize bacterial colonization in larvae with 16S-FISH and compare the bacterial microbiomes of larvae raised in ocean water to those of larvae exposed to adult-associated bacteria in the laboratory. This 'artificial' microbiome is similar to, but significantly less diverse, than its more natural counterpart. Regardless, bacteria-exposed larvae are significantly more resistant to the larva-associated pathogen *Vibrio lentus*, which induces a potentially lethal vibriosis by secretion and cleavage of the $Zn^{2+}$-dependent metalloprotease vibriolysin/Vsm. Other larva-associated bacterial isolates are benign or induce mild IL-17 responses in the midgut epithelium compared to *V. diazotrophicus*, but at least one *Colwellia* strain induces a novel pattern of IL-17-4 and IL-17-1 expression in cells around the mouth and throughout the blastocoel. We also discuss ongoing RNA-seq and ISH experiments to profile immune cell behaviour and immune gene expression during colonization by these strains. These results show that larvae recruit specific bacterial communities distinct from their environments, and that these communities influence development and immunity.

# SEARCHING FOR NK CELL-LIKE ACTIVATING AND INHIBITORY RECEPTORS IN THE MARINE INVERTEBRATE *BOTRYLLUS SCHLOSSERI*

Jonathan H. Schultz, Anthony De Tomaso

*Department of Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, Santa Barbara, CA 93106, USA*

The marine invertebrate *Botryllus schlosseri* has been and continues to be an important model for investigating immune function because of its ability to discriminate among hundreds of histocompatibility alleles in a natural population and partake in fusion/rejection reactions with neighboring colonies. As a member of the Urochordata (sister group to Vertebrata) it is presumed that key evolutionary hallmarks of vertebrate immunity also belong to *B. schlosseri* (among others). Recent evidence suggests that *B. schlosseri* harbors immune-cell types shared by a common ancestor of both phylogenetic groups. However, there is no current designation of vertebrate-like lymphocytes (e.g., NK cells) in *B. schlosseri*. To circumvent the challenges of identifying NK cell-like surface receptors using sequence homology-based methods, a "black box" bioinformatic approach was used to scan for conserved ITIM (S/I/V/LXYXXI/V/L) and ITSM (S/TXXYXXL/I) motif-containing sequences within a reference transcriptome of *B. schlosseri* generated from three publicly available transcriptomic datasets. For designation as a putative NK cell-like activating/inhibitory cell-surface receptor, sequences were required to contain: 1) a single transmembrane domain; 2) ITSM and ITIM motifs located within a cytoplasmic tail; 3) predicted central tyrosine phosphorylation and 4) upstream non-cytoplasmic protein domains. Among 194,037 translated nucleotide sequences, eight ITSM-containing sequences with upstream immunoglobulin domains were recovered. In addition, two ITIM-containing sequences with upstream CLECT domains and 15 ITIM-containing sequences with upstream immunoglobulin domains were also found. These results indicate the potential for an NK cell-like repertoire within *B. schlosseri* and experiments are underway to validate bioinformatic predictions and localize expression of these receptors.

# PACAP ACTS AS AN ANTIMICROBIAL PEPTIDE AND IMMUNOSTIMULANT DURING *FLAVOBACTERIUM PSYCHROPHILUM* INFECTIONS IN RAINBOW TROUT MACROPHAGES

Shawna L. Semple[1], Tania Rodríguez-Ramos[1], Yamila Carpio[2], John S. Lumsden[3], Mario P. Estrada[2] and Brian Dixon[1]

[1]Department of Biology, University of Waterloo, Waterloo, ON, Canada
[2]Center for Genetic Engineering and Biotechnology, Havana, Cuba
[3]Department of Pathobiology, University of Guelph, Guelph, ON, Canada

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional neuropeptide capable of performing roles as a neurotransmitter, neuromodulator and vasodilator. This polypeptide belongs to the glucagon/secretin superfamily, of which some members have been shown to act as antimicrobial peptides in both mammalian and aquatic organisms. In teleosts, PACAP has been demonstrated to have direct antimicrobial activity against several aquatic pathogens, yet this phenomenon has never been studied throughout a live bacterial infection. The present study focuses on the influence of PACAP on the rainbow trout monocyte/macrophage-like cell line, RTS11, when exposed to the coldwater bacterial pathogen *Flavobacterium psychrophilum*. PACAP was shown to have direct antimicrobial activity on *F. psychrophilum* when grown in both cytophaga broth and cell culture media. Further, the ability of teleostean PACAP to permeabilize the membrane of an aquatic pathogen, *F. psychrophilum*, was revealed for the first time. Interestingly, when RTS11 was pre-treated with PACAP for 24 hours before experiencing exposure to live *F. psychrophilum*, growth of the pathogen was severely inhibited in a dose-dependent manner. Relative expression of pro-inflammatory cytokines and PACAP receptors was also observed in RTS11 following PACAP exposure alone and in conjunction with live *F. psychrophilum* challenge. The results of this study provide evidence that PACAP has immunostimulatory activity on rainbow trout macrophages as well as direct antimicrobial activity against an aquatic bacterial pathogen. As there are numerous pathogens that impact the aquaculture industry, PACAP may stimulate the teleost immune system while also providing an efficacious alternative to antibiotic use.

# SITES OF HEMATOPOIESIS IN THE SEA URCHIN

Preethi Golconda, Katherine M. Buckley, Caroline R. Reynolds, Jennifer Romanello, L. Courtney Smith

*Department of Biological Sciences, George Washington University, Washington DC*

The location of coelomocyte proliferation in adult sea urchins is unknown although this question has been addressed repeatedly since the early 1800s. Immunoquiescent (IQ) purple sea urchins (*Strongylocentrotus purpuratus*) with down-regulated immune systems have reduced numbers of coelomocytes that increase quickly in response to immune challenge. Whether some or all of these cells are newly proliferated is not known. Furthermore, the gene regulatory network that regulates hematopoiesis in embryonic and larval sea urchins has not been investigated in adults. Hence, cell proliferation was induced in IQ sea urchins either by injection of heat-killed *Vibrio diazotrophicus* or by aspiration of coelomic fluid. In response, coelomocyte concentration increases, but newly proliferated coelomocytes constitute only about 10% of this cellular increase. In tissues, newly proliferated cells are present in the axial organ, gonad, pharynx, esophagus, and gut with no differences among tissues. Expression of genes encoding transcription factors that regulate hematopoiesis are elevated in the axial organ and the pharynx compared to coelomocytes, esophagus, gut, and gonad. This result is in agreement with an evaluation of an RNAseq dataset for adult sea urchin tissues, which also suggests that the axial organ is a center of apoptosis processes. Results indicate that the axial organ may be a site of coelomocyte proliferation and that it may also be a center for cellular removal and recycling. A second unexpected site, the pharynx, may also have hematopoietic activity, a tissue that has been assumed previously to function only as part of the intestinal tract.

# EFFECT OF TEMPERATURE ON WOUND HEALING IN GOLDFISH

Amro M. Soliman[1], Farah Haddad[2], Daniel R. Barreda[1,2]

[1] *Department of Biological Sciences, University of Alberta, Edmonton, Canada*
[2] *Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, Canada*

Wound healing is a complex biological process accomplished through the control of inflammation, homeostasis, and cellular proliferation. Our lab has established an important role of behavioral fever in the induction and resolution of inflammation. The aim of this study is to investigate the role of behavioral fever in the healing of infected wounds in goldfish. Goldfish infected *in vivo* with *Aeromonas veronii* (Gram-negative bacterium found in freshwater) were assigned to three distinct temperature categories: 1) static 16◦c; 2) static 25◦c; and 3) Dynamic temperature (allowing the fish to swim freely through different temperatures in an annular thermal preference tank) for 14 days. Video monitoring showed that infected fish spent the first 8 days at the highest temperature (25◦c) thermal compartment. Macroscopic analysis revealed that wound closure was faster in dynamic and 25◦c groups compared to 16◦c group. Histological analysis showed a rapid infiltration of inflammatory cells followed by a fast resolution in dynamic and 25◦c. Meanwhile, there was a delay in cellular infiltration to the wound site in 16◦c group. Moreover, the amount of collagen deposition in the wound area was more in the dynamic group. Also, the dynamic group showed early development of intact epidermis and dermis layers compared to other groups. Quantitative PCR analyses are ongoing for genes related to wound healing. We hypothesize that the expression of these genes will be upregulated in the dynamic group compared to other groups. This will be coupled to histological examination to assess the mechanisms driving wound healing during fish thermoregulatory responses.

**Discovery of cytotoxic killer IgT$^+$ B cells in fish**

Yasuhiro Shibasaki[1,2], Fumio Takizawa[1,3], Michael Gonzalez[4], Pierre Boudinot[5], J. Oriol Sunyer[1]

[1] Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania,
[2] JSPS, Overseas Research Fellow,
[3] Faculty of Marine Science and Technology, Fukui Prefectural University,
[4] Children's Hospital of Philadelphia,
[5] Virologie et Immunologie Moléculaires, INRA, Université Paris-Saclay

Our previous work has shown that IgT plays a key role in teleost fish mucosal immune responses. We have demonstrated that IgT is produced by a subset of B cells that uniquely express membrane IgT while lacking membrane IgD. In addition to playing a key role in adaptive mucosal immune responses, we have also shown an involvement of IgT$^+$ B cells in innate immunity as they possess a high phagocytic and microbicidal capacity. To understand further the roles of these cells in innate immunity, we performed the first comparative transcriptome analysis on FACS-sorted IgT$^+$ and IgM$^+$ B cells. To our surprise the gene that showed the highest differential expression between these two B cell subsets was perforin, a molecule that is not typically associated with B cells. The results obtained by transcriptome were expanded by further RT-PCR analysis confirming that unlike IgM$^+$ B cells, IgT$^+$ B cells expressed high transcript levels of several perforin isoforms, including, prf1-like-B, prf1-like-C and prf1-like-D. We also confirmed the unique expression of these perforin genes in IgT$^+$ B cell by single cell transcriptome analysis. Moreover, we produced antibodies against these perforin molecules and demonstrated by immunohistochemistry the presence of several of these perforin isoforms in IgT$^+$ B cells. Since perforin is a cytolytic protein that forms pores on the cell membrane of target cells, we hypothesized that IgT$^+$ B cells could possess cytotoxic activity similar to that of other perforin-expressing cells, including CD8$^+$ T cells and NK cells. To confirm this hypothesis, we tested the potential cytotoxic capacity of IgT$^+$ B cells towards several mammalian cell lines, such as HL-60. Our results show that the killing activity of IgT$^+$ B cells was significantly greater than that of IgM$^+$ B cells. These data demonstrate a previously unrecognized new function for IgT$^+$ B and vertebrate B cells in immunity.

# *EVOLUTIONARY CONSERVED MECHANISMS OF HEMATOPOIESIS, LESSONS FROM A COLONIAL CHORDATE*

**Ayelet Voskoboynik**

[1]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, CA USA. [2]Department of Biology, Stanford University, Hopkins Marine Station, CA , USA. [3]Chan Zuckerberg Biohub, San Francisco CA, USA.

Hematopoiesis is an essential process that evolved in multicellular animals. At the heart of this process are hematopoietic stem cells (HSCs) which are multipotent, self-renewing and generate the entire repertoire of blood and immune cells throughout life. While there are comprehensive studies on HSC self-renewal, differentiation, regulation and niche occupation, relatively little is known about the evolutionary origin of HSCs. We study the hematopoietic system of *Botryllus schlosseri*, a colonial chordate with vasculature containing circulating blood cells, and interesting characteristics of stem cell biology and immunity. Self-recognition between compatible colonies leads to formation of natural chimeras, whereas genetically incompatible colonies reject. This self–nonself recognition process is controlled by a highly polymorphic histocompatibility gene BHF: at least one shared BHF allele is required for fusion to take place (1). By comparing the *Botryllus* genome with those of a number of vertebrates, we identified multiple genes that may have contributed to the evolution of hematopoiesis (2). Using cell labeling, engraftment, confocal microscopy, and time-lapse imaging, we identified cells with stem capabilities in the anterior ventral region of the *Botryllus* endostyle (3). Based on whole-transcriptome sequencing of defined cell populations sorted by flowcytometry, and diverse functional assays, we further identified HSCs and demonstrated that the endostyle is a hematopoietic stem cell niche (4). This study revealed a significant evolutionary conservation between the gene repertoire of the *Botryllus* and mammalian hematopoietic stem cells (HSC). The endostyle molecular signature further suggests that the vertebrate's hematopoietic bone marrow niche evolved from an organ resembling the *Botryllus* endostyle.

1. Voskoboynik A, Newman AM, Corey DM, Sahoo D, Pushkarev D, Neff NF, Passarelli B, Koh W, Ishizuka KJ, Palmeri KJ, Dimov IK, Keasar C, Fan HC, Mantalas GL, Sinha R, Penland L, Quake SR, Weissman IL. Identification of a colonial chordate histocompatibility gene. ***Science***. 2013 Jul 26; 341(6144): 384-387. PMCID: PMC3810301.

2. Voskoboynik A, Soen Y, Rinkevich Y, Rosner A, Ueno H, Reshef R, Ishizuka KJ, Palmeri KJ, Moiseeva E, Rinkevich B, Weissman IL. Identification of the endostyle as a stem cell niche in a colonial chordate. ***Cell Stem Cell***. 2008 Oct 9; 3(4): 456-464. PMCID: PMC4126901.

3.Voskoboynik A, Neff NF, Sahoo D, Newman AM, Pushkarev D, Koh W, Passarelli B, Fan HC, Mantalas GL, Palmeri KJ, Ishizuka KJ, Gissi C, Griggio F, Ben-Shlomo R, Corey DM, Penland L, White RA, Weissman IL, Quake SR. The genome sequence of the colonial chordate, *Botryllus schlosseri*. ***Elife.*** 2013 Jul 2; 2: e00569. PMCID: PMC3699833.

4. Rosental B, Kowarsky MA, Neff NF, Corey DM, Ishizuka KJ, Palmeri KJ, Chen SY, Sinha R, Newman AM, Clarke ND, Seita J, Mantalas GL, Okamoto J, Quake SR, Weissman IL Voskoboynik A Complex Mammalian-like Hematopoietic System Found in a Colonial Chordate **Nature** 2018 Dec 5 doi:10.1038s41586-018-0783-x.

**Title: Identification, characterization, and expression analysis of goldfish (*Carassius auratus*) leukocyte immune-type receptors**

Jiahui Wang, James L. Stafford, and Miodrag Belosevic

Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada

Teleost Leukocyte immune-type receptors (LITRs) represent a unique family of immune proteins composed of multiple extracellular Ig-like protein domains homologous to both mammalian FcRs, FCRLs, and receptors encoded in the leukocyte receptor complex. Although a considerable amount of information on the biochemical and functional potentials of catfish LITRs (IpLITRs) have been gained by expressing IpLITRs in mammalian cell lines, we know very little regarding their immunoregulatory roles in fish immune cell-types. In this study, we identified and cloned three goldfish (*Carassius auratus*) LITR-types, CaLITR1, 2, and 3. All three CaLITRs share significant sequence homology with IpLITRs and are related to mammalian FCRLs. CaLITR1 and 3 appear to be putative stimulatory receptors containing positively charged transmembrane domains, and short cytoplasmic regions devoid of obvious signaling motifs. Conversely, CaLITR2 has a neutral transmembrane domain, and a long cytoplasmic tail containing an ITAM-like motif. We examined the expression of CaLITRs in goldfish tissues (liver, spleen, heart, kidney, brain, intestine, gill, and muscle) and during primary kidney macrophage (PKM) development. Results show that CaLITRs are broadly and differentially expressed in goldfish tissues and are switched on early during PKM development. Examination of CaLITRs in goldfish PKM and PKNs is an important step towards understanding their immunoregulatory roles in fish.

# NOVEL ALLELES OF ANTIGEN PROCESSING GENES IN RAY-FINNED FISHES

Dustin J. Wcisel[1], Alex Dornburg[2], Andrew Thompson[3], Ingo Braasch[3], and Jeffrey A. Yoder[1]

1. Department of Molecular Biomedical Sciences, North Carolina State University, Raleigh, NC USA
2. North Carolina Museum of Natural Sciences, Raleigh, NC USA
3. Department of Integrative Biology, Michigan State University, Lansing, MI USA

The inducible proteasome subunit (PSMB) genes are part of the 20S proteasome complex, responsible for systematically degrading proteins into small polypeptides for loading and display on major histocompatibility (MHC) proteins. In the genomes of teleost species such as zebrafish, the PSMB genes are clustered together adjacent to their MHC Class I counterparts. Within a given species, alternative haplotypes for these genes have been described. For example, the 31$^{st}$ amino acid residue of the PSMB8 mature protein is found to contain either an alanine or a phenylalanine (PSMB8A or PSMB8F, respectively). The sequence differences may lead to functional changes in the types of proteins degraded and thus the antigens displayed. These alternative alleles are present in Senegal bichir (*Polypterus senegalus*), and numerous teleost species as well as many tetrapods (*Xenopus tropicalis* for example). This type of conserved haplotypic variation is referred to as trans-species polymorphism. Recently, the genome sequencing of two holostean species, spotted gar (*Lepisosteus oculatus*) and bowfin (*Amia calva*) were completed. Here, we present the annotation of the PSMB loci in both species using the zebrafish genome as a reference point. We found the holostean PSMB8 gene, while highly conserved with other fish species, encodes novel alleles containing either a threonine or serine at the 31$^{st}$ residue. Many additional polymorphisms were also identified in the neighboring genes, suggesting holosteans experienced unique co-evolution of these genes at this locus likely resulting in functional differences of antigen presentation.

# DOES THERMAL PRECONDITIONING DURING DEVELOPMENT ALTER THE STRESS AND IMMUNE RESPONSES OF JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*) EXPOSED TO A THERMAL CHALLENGE?

Lindy Whitehouse[1], H. Guo[1], M. Ferguson[2], R. Danzmann[2] and B. Dixon[1]

[1] University of Waterloo, Waterloo, ON.

[2]University of Guelph, Guelph, ON.

* Email: lindy.whitehouse@uwaterloo.ca

Climate change is the leading threat facing Canadian Aquaculture, with increases in a plethora of stressors that can have long-lasting damaging effects. Of these stressors, increasing temperature is perhaps the most predominant threat, causing reduced feeding and an increase in susceptibility to disease as a result of stress. This increase in stress results in a decrease in the health of the fish, and from an aquaculture perspective, a decrease in productivity. One of the methods suggested to mitigate the stress associated with increasing temperatures is thermal preconditioning. To assess the impact of thermal preconditioning on the stress response and immune responses of rainbow trout (*Oncorhynchus mykiss*), we exposed developing rainbow trout to spikes in temperatures twice a week for six weeks after the swim-up stage before exposing them to a thermal challenge. Responses were compared between a control group, a low thermal tolerance and a high thermal tolerance group for both control and preconditioned fish. Results show that preconditioning results in altered stress and immune responses in the spleen at the mRNA level for *hsp70*, *il-1β* and *il-6* but only for fish in the lower thermal tolerance group. Further, preconditioning had no effect on the mRNA levels of *tnfα*. This presentation will also explore protein levels for the aforementioned genes. The outcomes of this study could establish the thermal reconditioning method as a viable means to improve overall fish health which could mitigate some of the threat that climate change poses to Canadian aquaculture and increase aquaculture productivity.

# UNDERSTANDING THE MOLECULAR DIVERSIFICATION OF SELF RECOGNITION THROUGH RAY-FINNED FISH INNATE IMMUNE RECEPTOR FAMILIES.

Dustin J. Wcisel[1], Jeffrey A. Yoder[1,2,3], Lindsay Roupe-Abrams[4], Alex Dornburg[4]

*1. Department of Molecular Biomedical Sciences, 2. Comparative Medicine Institute, 3. Center for Human Health and the Environment, NC State University, Raleigh, NC USA*
*4. North Carolina Museum of Natural Sciences, Raleigh, NC USA*

The use of molecular markers of self-identity as a basis for immunity was a major evolutionary innovation in the early history of vertebrates. The ability to discriminate between self and non-self has since spurred a coevolutionary competition between hosts and pathogens driving high levels of both inter- and intraspecific immune gene sequence diversity. Such diversification is essential for a species to survive new pathogens, yet the origin and evolutionary dynamics of vertebrate self recognition remain poorly understood. A powerful system for understanding the genetic and functional evolution of immune genes associated with self recognition are ray-finned fishes (*Actinopterygii*) which constitute over half of all living vertebrates. Like all vertebrates, fish possess certain core immune gene families, however they also encode a number of "fish-specific" immune gene families. Using a phylogenetic comparative framework, we integrate transcriptome and genomic sequence data from early diverging lineages of ray-finned fishes to establish the evolutionary origins of fish-specific immune receptor genes and test fundamental expectations of coevolution between markers of self and their candidate receptors. Our work provides a new perspective on the early history of the vertebrate immune system, overturning assumptions of teleost specific innovations, while revealing novel molecular innovations to pathogen resistance.