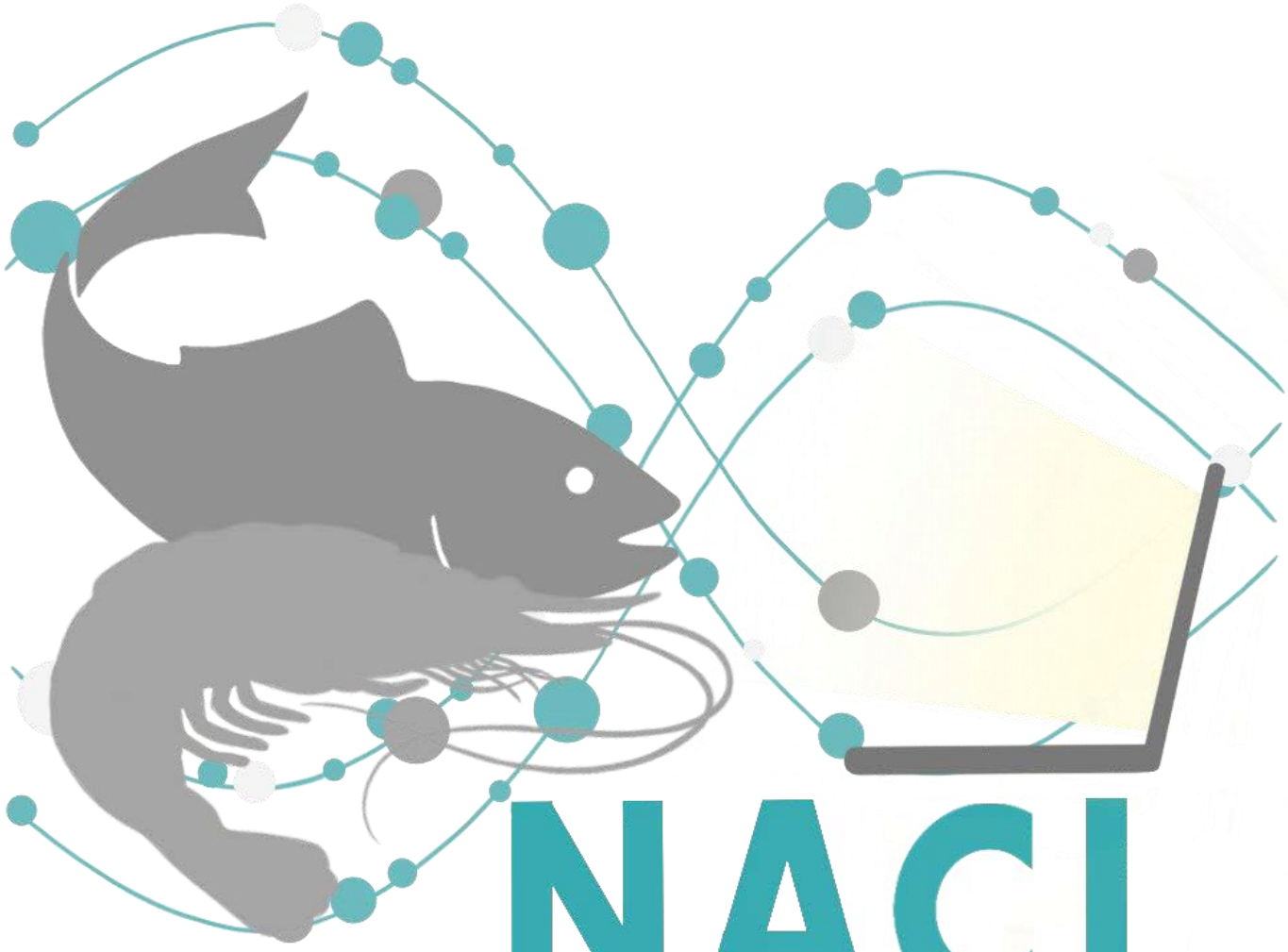

NACI WORKSHOP 2021

June 8th to 10th

Schedule

Time Zone:		PST	MST	CST	EST	AST	ZOOM INFO	Host
Session 1: **Invertebrate Immunity**	08-Jun	8:00 AM	9:00 AM	10:00 AM	11:00 AM	12:00 PM	https://unm.zoom.us/j/95475037297 Meeting ID: 954 7503 7297 Passcode: 183767	Dixon
Session 2: **Innate Immunity**	08-Jun	10:00 AM	11:00 AM	12:00 PM	1:00 PM	2:00 PM	https://unm.zoom.us/j/95879261048 Meeting ID: 958 7926 1048 Passcode: 183767	Magor
Session 3: **Fish Innate immunity**	08-Jun	1:00 PM	2:00 PM	3:00 PM	4:00 PM	5:00 PM	https://unm.zoom.us/j/91657648241 Meeting ID: 916 5764 8241 Passcode: 183767	Salinas
Happy Hour I	08-Jun	2:30 PM	3:30 PM	4:30 PM	5:30 PM	6:30 PM	https://unm.zoom.us/j/98161896298 Meeting ID: 981 6189 6298 Passcode: 183767	
Session 4: **Amphibian innate Immunity**	09-Jun	8:00 AM	9:00 AM	10:00 AM	11:00 AM	12:00 PM	https://unm.zoom.us/j/93653463914 Meeting ID: 936 5346 3914 Passcode: 316017	Grayfer
Session 5: **MHC and Evolution**	09-Jun	10:00 AM	11:00 AM	12:00 PM	1:00 PM	2:00 PM	https://unm.zoom.us/j/92936033077 Meeting ID: 929 3603 3077 Passcode: 316017	Robert
Happy Hour II	09-Jun	1:00 PM	2:00 PM	3:00 PM	4:00 PM	5:00 PM	https://unm.zoom.us/j/96202881910 Meeting ID: 962 0288 1910 Passcode: 316017	
Session 6: **Ig superfamily receptor evolution**	10-Jun	8:00 AM	9:00 AM	10:00 AM	11:00 AM	12:00 PM	https://unm.zoom.us/j/92733463263 Meeting ID: 927 3346 3263 Passcode: 467993	Yoder
Session 7: **B cell function and evolution**	10-Jun	10:00 AM	11:00 AM	12:00 PM	1:00 PM	2:00 PM	https://unm.zoom.us/j/96875455034 Meeting ID: 968 7545 5034 Passcode: 467993	Buckley
Happy Hour GALA Report on NACIW 2022	10-Jun	1:00 PM	2:00 PM	3:00 PM	4:00 PM	5:00 PM	https://unm.zoom.us/j/93609196324 Meeting ID: 936 0919 6324 Passcode: 467993	

Program at a glance

Tuesday June 8th, 2021		
Session 1: Invertebrate Immunity	Eliachar S	Functional characterization of Hexacorallian phagocytic cells
Host: Brian Dixon	Singh S	The central role of phagocytes in two opposing vascular remodeling events: vascular regression and vascular regeneration in *Botryllus schlosseri*
https://unm.zoom.us/j/95475037297 Meeting ID: 954 7503 7297 Passcode: 183767	Kowarsky MA	SEXUAL AND ASEXUAL DEVELOPMENT: TWO DISTINCT PROGRAMS PRODUCING THE SAME TUNICATE
	Yakovenko I	Diverse Trf protein family in the sea urchin *Paracentrotus lividus* acts through collaboration between cellular and humoral immune effector arms
	Monod E	Characterizing the response of aquatic invertebrates to immune stimulation by dsRNA and PACAP-38
	Rodriguez Valbuena H	Evolution of *B. schlosseri* allorecognition genes
Session 2: Innate Immunity	Prigozhin DM	A limited subset of plant immune receptors is responsible for generating new specificities.
Host: Kathy Magor	Moghrabi A	Inhibition of MAVS Signaling by PB1-F2 from H5N1 Highly Pathogenic Avian Influenza Viruses in Avian Cells
https://unm.zoom.us/j/95879261048 Meeting ID: 958 7926 1048 Passcode: 183767	Campbell LK	The duck TRIM gene repertoire is differentially expressed in response to highly pathogenic H5N1 influenza A virus
	Bakke FK	Comparative analysis of the plasma proteomes of distantly related vertebrates
	He W	Development of a natural model of necrotic enteritis to examine immunomodulation in poultry
	Soliman AM	Contributions of Fever to Tissue Repair
	Semple SL	Novel use of long dsRNAi as an antiviral therapy across vertebrate species.
Session 3: Fish Innate immunity	Mani A	Regional immune responses in the central nervous system of rainbow trout to systemic commensal bacteria
Host Irene Salinas	Fajei E	Boosting tilapia (*Oreochromis niloticus*) immune responses to *Flavobacterium columnare* using PACAP (pituitary adenylate cyclase activating polypeptide)
https://unm.zoom.us/j/91657648241 Meeting ID: 916 5764 8241 Passcode: 183767	Groves L	The effects of climate change on the Atlantic salmon's (*Salmo salar*) immunological response to infectious salmon anemia (ISAv)
	Samms KA	DsRNA Formulated with Phytoglycogen Nanoparticles to Induce Innate Immune Responses in Rainbow Trout and Prevent Viral Replication of ISAV in Atlantic Salmon
	Rodriguez Cornejo T	Pituitary adenylate cyclase-activating polypeptide (PACAP) as a prophylactic alternative against bacterial infections in rainbow trout (*Oncorhynchus mykiss*).

	Frenette AP	Developing reagents for assessing protein-level changes for cellular and oxidative stress responses in cultured Atlantic salmon (*Salmo salar*) in the face of temperature and hypoxia challenge
	Niemand RR	Examination of a New Family of Fish Immune-Type Receptors may Reveal Novel Insights into the Mechanisms Controlling Vertebrate Innate Immunity
		https://unm.zoom.us/j/98161896298 Meeting ID: 981 6189 6298 Passcode: 183767
Wednesday June 9th, 2021		
Session 4: Amphibian innate Immunity Host: Leon Grayfer https://unm.zoom.us/j/93653463914 Meeting ID: 936 5346 3914 Passcode: 316017	Rollins-Smith LA	Amphibian Phagocyte Functions are Inhibited by the Deadly Chytrid Fungus, *Batrachochytrium dendrobatidis*
	Todd LA	microRNAs and the regulation of innate antiviral immunity in frogs
	Hauser K	Just a gut feeling: Amphibian (*Xenopus laevis*) tadpoles and adult frogs mount distinct responses to intestinal Frog Virus 3 infections
	Hossainey MRH	The roles of amphibian (*Xenopus leavis*) macrophage subsets during chronic Frog Virus 3 infections
	Dimitrakopoulou D	A novel *Xenopus laevis* model to evaluate the pathogenicity dissemination, and resistance of *Mycobacterium abscessus.*
	McGuire CC	Thyroid Disrupting Chemicals Perturb Thymocyte Development in *Xenopus laevis*
Session 5: MHC and Evolution Host: Jacques Robert https://unm.zoom.us/j/92936033077 Meeting ID: 929 3603 3077 Passcode: 316017	Sultan E	Histocompatibility response in Planaria and its mediation by cellular immune activity
	Carlson K	Major histocompatibility complex as an indicator of divergence in longnose gar
	Almeida T	Eight MHC class I lineages in the oldest vertebrates with human-like adaptive immunity
	Veríssimo A	Cartilaginous fish class II genes reveal 350 My-old allelic lineages in sharks but no DM
	Paiola M	Deciphering the role of MHC class I-like XNC4 interacting T cells during mycobacterial infection in *Xenopus*
	Lopez V	Cell surface expression of the non-polymorphic MHC class I-like molecule XNC4 does not strictly require b2-microglobulin association
	Sampson JM	Olfactory receptors on lymphocytes in a marsupial
Happy Hour II 		https://unm.zoom.us/j/96202881910 Meeting ID: 962 0288 1910 Passcode: 316017

Thursday June 10th, 2021		
Session 6: Ig superfamily receptor evolution Host: Jeff Yoder https://unm.zoom.us/j/92733463263 Meeting ID: 927 3346 3263 Passcode: 467993	Morrissey KA	The third domain of T cells: Defining the uniquely mammalian γμ T cell
	LeNours J	Structural characterization of the marsupial γμ T cell receptor
	Wcisel DJ	A highly diverse set of novel immunoglobulin-like transcript (NILT) genes in zebrafish indicates a wide range of functions
	Wang J	Expression analyses of CaLITRs in response to various immune stimuli
	Kyslík J	The evolutionary history of receptors for immunoglobulins reveals the origin and the complexity of adaptive immune systems at the base of tetrapod evolution
	Majstorović J	Expression of putative Fc receptors in different immune cell types of the rainbow trout (*Oncorhynchus mykiss*)
Session 7: B cell function and evolution Host: Kate Buckley https://unm.zoom.us/j/96875455034 Meeting ID: 968 7545 5034 Passcode: 467993	Matz H	Evolutionary Precursors of Germinal Centers are Present in Sharks
	Chan JTH	Ab-normal erythrocytes in proliferative kidney disease (PKD) – rainbow trout (Oncorhynchus mykiss) infected by *Tetracapsuloides bryosalmonae* harbor IgM+ red blood cells
	Waly D	Antibody affinity modification in fish
	Attaya A	Single cell-RNA seq profiling of spleen cells in naïve and immunized threespine stickleback fish (*Gasterosteus aculeatus)*
	Yakovenko I	Guardian of the genome: the prequel to V(D)J recombination by RAGs
	Barker A	Proteasome subunit 8 (PSMB8) of ray-finned fishes; cataloging trans-species polymorphisms
Happy Hour GALA 		https://unm.zoom.us/j/93609196324 Meeting ID: 936 0919 6324 Passcode: 467993

Abstracts

Session 1: Invertebrate Immunity

Functional characterization of Hexacorallian phagocytic cells

Eliachar S.[1]; Snyder GA.[2]; Gershoni-Yahalom O.[1]; Traylor-Knowles N.[2]; Rosental B[1].

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[2] *Department of Marine Biology and Ecology, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL, USA*

Abstract: Phagocytosis is a fundamental cellular immune mechanism in defending animals against various pathogens. Therefore, phagocytosis is a pillar of innate immunity, whereby foreign particles or infected cells are engulfed and degraded in lysolitic vesicles. Additionally, phagocytosis is the mechanism of clearing damaged cells to enable tissue regeneration. In hexacorallians, phagocytic mechanisms are poorly understood. In the current work, we characterize phagocytes on the functional cellular level from two hexacorallian species, the coral *Pocillopora damicornis*, and the anemone *Nematostella vectensis*. We show that distinct populations of phagocytic cells engulf bacteria and carboxylated beads. We demonstrate that this phagocytosis is different from small molecule pinocytosis. Inhibiting actin filament rearrangement interferes with efficient particle phagocytosis but does not affect small molecule pinocytosis. We show the internalization of the engulfed particles and their fusion with lysolitic vesicles using confocal microscopy and ImageStream. We also demonstrate that markers for lysolitic vesicles and reactive oxygen species can be used as sorting enrichment markers for the phagocytes. These results establish a foundation for improving our understanding of hexacorallian immune cell biology.

The central role of phagocytes in two opposing vascular remodeling events: vascular regression and vascular regeneration in *Botryllus schlosseri*

Singh Shambhavi1; De Tomaso, Anthony W.1

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Phagocytes are an essential group of cells in the mammalian immune system responsible for causing and resolving inflammation. The role of phagocytic cells, particularly mammalian tissue-resident macrophages, in regenerative processes have been explored in great detail in recent years. However, the molecular orchestration of the phagocytes' dual function in both cytotoxicity and regeneration is not well understood. Our research's primary goal is to determine the molecular mechanisms controlling inflammatory and regenerative functions of phagocytic cells. The basal chordate *Botryllus schlosseri* is an excellent model for these studies. Botryllus has an extensive, transparent extracorporeal vasculature, which is ideal for visualizing, quantifying, and isolating blood cells. We are studying two opposing vascular remodeling events: vascular regeneration and regression. Following partial ablation of the vasculature, the vessels will regenerate relatively at a slower rate. In contrast, vascular regression is an inducible remodeling event during which phagocytic cells migrate to and remove apoptotic cells from the blood vessel wall, causing vessel shortening. Hence, ablation of the phagocytes reduces the rate of regression of the vasculature. Along with ablation studies to characterize phagocyte dynamics during regeneration and regression, we will also perform bulk single-cell RNA sequencing on phagocytes during these two processes. It will equip us to perform differential gene expression analysis to better understand phagocyte dynamics during regeneration and regression. The results will point towards the specific molecular patterns that are associated with opposing behaviors in phagocytes. Finally, single-cell sequencing will provide a higher resolution of cellular differences for a better understanding of individual macrophages' function in the context of their microenvironment.

SEXUAL AND ASXUAL DEVELOPMENT: TWO DISTINCT PROGRAMS PRODUCING THE SAME TUNICATE

Kowarsky MA[1], Anselmi C[2,3,4], Hotta K[5], Burighel P[2], Zaniolo G[2], Caicci F[2], Rosental B[3,4,6], Neff NF[7], Ishizuka KJ[3,4], Palmeri KJ[3,4], Okamoto J[7], Gordon T[8] Weissman IL[3,4,7], Quake SR[6,7,8], Manni L[2], Ayelet Voskoboynik[3,4,6].

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This study presents a thorough molecular and morphological characterization of two distinct developmental pathways in the colonial chordate *Botryllus schlosseri*. Colonial tunicates, like *B. schlosseri* are unique amongst chordates in possessing two distinct developmental pathways that produce the adult body, either sexually through embryogenesis, or through a stem cell mediated asexual renewal termed blastogenesis. Through embryogenesis a larva is developed possessing chordate structures such as a notochord, neural tube, segmented musculature, tail, photolith, and larval brain. The hatched larva swims to subtidal surfaces, where it settles and metamorphoses into its invertebrate stage body, the zooid. Meanwhile the zooid's buds undergo blastogenesis, developing new zooids without an embryonic intermediate. Using this model organism, we have combined transcriptome sequencing of major embryonic and blastogenic stages and multiple tissues and enriched stem cell populations with confocal, two-photon and electron microscopy to characterize the molecular and morphological signatures along both developmental pathways. We developed a novel computational pipeline to comprehensively identify gene expression patterns across all sets of contiguous developmental time points. Per every embryonic or blastogenic developmental stage, we identify unique and shared molecular characteristics. These analyses revealed that while the overall molecular programs are distinct, the blastogenic tissue-specific stem cells and embryonic precursor populations share similar molecular profiles. To generate a quantitative understanding of how a set of genes (e.g. specific cell population signature) is enriched at different time points across the developmental pathways, we also identified the molecular signatures of specific tissues and enriched stem cell populations and use it to track the developmental origin of hematopoiesis, germ cells and central nervous system organogenesis along the developmental stages. This work demonstrates the extent to which convergent morphology implies convergent molecular mechanisms by combining microscopy with transcriptome sequencing and reveals the basic principles and evolutionary conserved elements of chordate development. It also uncovered the stages when tissue specific stem and progenitor precursor cells emerge in both developmental pathways, suggesting a link between embryonic and adult tissue specific stem cells.

Diverse Trf protein family in the sea urchin *Paracentrotus lividus* acts through collaboration between cellular and humoral immune effector arms

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Abstract: Sea urchins are long-living invertebrates with a complex and robust immune system that includes extended gene families of immune receptors. One of the central immune gene families in the sea urchin immune response encodes for Transformer (Trf) proteins. The *Trf* gene family is regarded as echinoid-specific and was extensively studied in the California purple sea urchin *Strongylocentrotus purpuratus*. Trf proteins selectively bind to different antigens, participate in the opsonization of bacteria, augment phagocytosis and retard bacterial growth. In this study, we explore the cellular and humoral effector arms of the Trf protein family in the Mediterranean sea urchin *Paracentrotus lividus*. The *PlTrf* genes and the predicted proteins were highly diverse and showed a typical Trf size range and structure. We found that the cellular and the humoral coelomic fluid fractions have differences in PlTrf protein profiles but share a specific PlTrf protein profile when bound to *E.coli*. Using FACS, we isolated at least five different *P. lividus* coelomocyte populations with membranal Trf expression. The relative expression of the Trf-expressing cells sharply increased following immune challenge with *E. coli* bacteria, but not following challenge with *P. lividus* pathogen *V. penaeicida* or with LPS from *E. coli*. Finally, we demonstrated that the phagocytosis of *E. coli* bacteria by *P. lividus* phagocytes is mediated through the hemolymph and may be inhibited by blocking Trf activity using an anti-Trf antibody. Our results demonstrate the collaboration of cellular and humoral Trf-mediated effector arms in the *P. lividus* immune response to pathogens.

Characterizing the response of aquatic invertebrates to immune stimulation by dsRNA and PACAP-38

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

Abstract: The fast-paced industry expansion of shrimp aquaculture has been accompanied by several catastrophic viral shrimp pandemics. Shrimp farmers are limited in their ability to manage and reduce disease burden due to the absence of effective antiviral therapies. The present study focuses on inducing a general immune response that will control viral infections in aquatic invertebrates. Double-stranded (ds)RNA has been well characterized in vertebrates as an innate immune stimulant and potent inducer of the antiviral response. Pituitary adenylate cyclase activating polypeptide (PACAP) is a highly conserved, multifunctional neuropeptide with antimicrobial properties. In vertebrates PACAP has been shown to regulate pro- and anti-inflammatory cytokine production through cAMP signalling cascades and to interfere directly with viral protein transcription. In this study wild caught native Ontario crayfish were injected with Polyinosinic:polycytidylic acid (synthetic dsRNA) and PACAP-38 and immune system activation was investigated between 6 hours and 7 days using functional markers. Initial findings demonstrate both stimulants induce immune pathways. Following *in vivo* stimulation, treated animals had higher total hemocyte count, improved lectin and nitric oxide metabolite generation as well as increased phenoloxidase concentration in the hemolymph. PACAP-38 also enhanced phenoloxidase production in the hemocytes between 6 and 24 hours. This work provides a fundamental understanding of the positive immuno-modulatory role of dsRNA and PACAP-38 in invertebrate species which will have implications for the shrimp aquaculture industry by contributing to the development of effective broad-spectrum immune therapies.

Evolution of *B. schlosseri* allorecognition genes

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Abstract

Allorecognition is the ability to distinguish between self and non-self tissues, which is present in variety of organisms, such as fungi, plants, and animals. In humans the allorecognition response is relevant in tissue and organ transplants, where the genes of the Major histocompatibility complex (MHC) control the process. By contrast, the allorecognition response occurs naturally in the urochordate *Botryllus schlosseri*, where colonies can fuse or reject each other. In this species the allorecognition is controlled by a single highly polymorphic locus called Fusion/histocompatibility (FuHc). Within this locus has been isolated at least six genes (*hsp40*, *bhf*, *fuhc-sec*, *fuhc-tm*, *fester* and *uncle fester*) related with the allorecognition response in *B. schlosseri*. The molecular mechanism behind these genes that explains the allorecognition response in *B. schlosseri* is under research. On other hand, we do not know if these allorecognition genes are present in other species of tunicates different to *B. schlosseri*. To solve this question, we analysed different transcriptomes and genomes of tunicates, and we identified the possible homologous of the allorecognition genes. We found that the *hsp40*, *bhf*, *fuhc-tm* and *fuhc-sec* genes are present in different species of tunicates, including species in which the allorecognition response is absent. This result indicates that these genes could are performing a different function to allorecognition in other tunicates species. By contrast, we found that the *fester* and *uncle fester* genes are present only in species that have the allorecognition response. Furthermore, we established that the genomic region that control allorecognition in *B. schlosseri* has experienced different genomic rearrangements during evolution. Finally, we are analysing the polymorphism of the allorecognition genes in other species of tunicates different to *B. schlosseri*. This analysis will allow to give an idea of the function of these genes in species that do not have the allorecognition response but have the allorecognition genes.

Session 2: Innate Immunity

A limited subset of plant immune receptors is responsible for generating new specificities.

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Plants rely on Nucleotide-Binding Leucine-Rich Repeat (NLR) immune receptors to detect presence of pathogen molecules in the cell cytoplasm. Within a single polypeptide, many NLRs encode functional recognition, oligomerization, and signaling modules. Our analysis of NLR variability within species, enabled by pan-genome and pan-NLRome sequencing, revealed a striking difference in the amount of sequence diversity observed within various NLR subfamilies.

In the model plant Arabidopsis thaliana, the highly variable NLR subfamilies included direct recognition receptors that bind to pathogen molecules, while low variability NLRs included indirect recognition receptors that detect pathogen-induced modification of plant proteins. The highly variable NLR subfamilies also contained the known autoimmune NLR alleles, consistent with their proposed role in generating novel specificities. Importantly, the highly variable subfamilies were distributed across the NLR phylogeny, suggesting a recurring evolutionary pattern of generation of receptor diversity coupled to preservation of successful receptor variants. Analyses of NLR sequences in non-model plants showed a similar pattern enabling functional inference based on observed sequence diversity.

In the highly variable receptor subfamilies, the observed sequence diversity was sufficient to predict their target-binding sites. We validated these predictions with targeted mutagenesis of a receptor of unknown structure and the analysis of a recently published structure of a receptor-target complex. We are currently investigating the genomic correlates and mutational profiles of the highly variable receptor families, as no mechanisms are known for targeted sequence diversification in the plant innate immune system.

Inhibition of MAVS Signaling by PB1-F2 from H5N1 Highly Pathogenic Avian Influenza Viruses in Avian Cells

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Upon detection of viral RNA, RIG-I engages the critical adaptor protein mitochondrial antiviral signalling (MAVS) to activate the downstream signalling pathway, leading to Type I interferon production. The influenza A virus non-structural protein, PB1-F2, interacts with MAVS in human cells to inhibit interferon production. We have shown in previous experiments that PB1-F2 from influenza virus A/Puerto Rico/8/1934 (H1N1) (PR8) will inhibit interferon production in avian cells. However, it is not known if this is true for highly pathogenic avian influenza A strains. In 2004, two highly pathogenic strains of H5N1 emerged in Southeast Asia; A/duck/D4AT/2004 (D4AT) and A/Vietnam/1203/04 (VN1203). These strains differ in virulence due to differences in PB1-F2 sequences. Here we use a Dual-Luciferase® Reporter Assay to show that PB1-F2 from highly virulent avian influenza A strains, as well as a reverse-genetics recombinant of VN1203 (rgVN1203), significantly inhibit production of IFN-β in chicken cells (DF-1). Using confocal microscopy, we show that PB1-F2 from H5N1 strains co-localize with duck MAVS. We also showed by Western Blot that PB1-F2 is immunoprecipitated by duck MAVS. This research highlights the mechanism by which PB1-F2, across various human and avian influenza strains, and provides a possible anti-viral therapeutic target.

The duck TRIM gene repertoire is differentially expresssed in response to highly pathogenic H5N1 influenza A virus

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Abstract: Tripartite motif (TRIM) genes are an ancient family of genes which has greatly expanded and diversified throughout vertebrate evolution. TRIM proteins have various functions from controlling general cellular processes to immunity, including restricting influenza A virus (IAV). Ducks are the natural host and reservoir of IAV, and as such likely have evolved many strategies to control the virus. It is currently unknown which TRIM genes are present in the duck TRIM repertoire, and how many of these genes are involved in IAV resistance or restriction. To determine which TRIM genes are present in the duck the NCBI SRA database was mined for RNA sequencing projects and a *de novo* assembly of the duck transcriptome was performed. From this transcriptome, 50 TRIM or TRIM-like genes were found to be present in the duck. To determine which of these genes may be involved in IAV restriction in the duck, I used RNA-seq to examine the expression patterns of TRIM genes ducks infected with a highly-pathogenic H5N1 strain of IAV (VN1203) or a low-pathogenic strain of H5N2 (BC500). VN1203 replicates in lungs of infected birds while BC500 replicates in intestines. My analysis found that VN1203 differentially expressed 31 of duck TRIM genes in lungs, and 23 of these genes in spleen. BC500 had much less of an effect on duck TRIM gene regulation with 7 of these genes differentially expressed in lung and spleen. While intestines of BC500 ducks do have a robust change in gene expression during infection, only 10 genes of the duck TRIM repertoire were differentially expressed. By examining expression of the TRIM gene repertoire in ducks infected with IAV, we can gain insight into the evolution TRIM genes in vertebrates and find candidate genes for IAV restriction in its natural host and reservoir.

Comparative analysis of the plasma proteomes of distantly related vertebrates

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[4] Institute of Marine and Environmental Technology (IMET), University of Maryland School of Medicine, Baltimore, USA

Abstract: The blood plasma proteome is poorly characterized in most vertebrates outside mammals, leaving a gap in our understanding of immunity and other biological processes influenced by circulating proteins. Here we present the first detailed comparison of the baseline plasma proteome of three basal vertebrate lineages, the rainbow trout, nurse shark, and sea lamprey. Empirical plasma proteome measurements were supported by inferences of gene homology relationships in addition to gene set enrichment analyses. While many of the proteins (or their inferred contribution to broader biological functions) show a degree of evolutionary conservation, taxa-specific differences in plasma protein presence and abundance were identified. For example, while putative orthologues of apolipoprotein B-100 were present at high abundance in all three species, only rainbow trout plasma showed high abundances of many other apolipoprotein family members. Further, two distinct forms of serum albumin were detected at high abundances in trout and lamprey plasma, but neither were identified in nurse shark. Interrogation of all available genetic data for Chondrichthyans also failed to detect any albumin-like molecules in this taxon. We also found that many of the proteins conserved across the three species were found at very different abundances, suggesting a degree of functional redundancy or that different proteins have evolved to support related processes through convergence. Overall, our study provides novel insights into the functional evolution of proteins involved in immunity and other biological processes.

Development of a natural model of necrotic enteritis to examine immunomodulation in poultry

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[2] *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada*
Abstract:

Necrotic enteritis (NE) is an economically important disease in poultry, caused by *Clostridium perfringens*. Colonization by *C. perfringens* occurs early during hatch, and induces host immune tolerance that allows it to persist as part of the bird's commensal flora. Conventional NE models typically employ exogenous inoculation with *C. perfringens* in excess to drive infection, which does not recapitulate the long-standing bacterial tolerance, microbial loads, and other relevant physiological factors that contribute to this disease in commercial production settings. Our goal was to establish a more representative model by taking advantage of classical conditions encountered in a commercial barn. In this study, we incorporated access to litter, coccidial exposure, feed composition, and feeding stress. Our model reproduced NE infection typically encountered in the field, where disease develops at week 3 post hatch. We then applied this model to evaluation for the capacity of β-glucan to reduce the negative impact of a NE challenge. Early age exposure to *C. perfringens* without β-glucan resulted in compromised feed conversion during the 40-day rearing period, which was avoided by β-glucan inclusion. qPCR and imaging flow cytometry analyses revealed enhanced antimicrobial parameters when animals were co-stimulated with β-glucan. Our results suggest that β-glucan promote long-term protection against *C. perfringens* by influencing the context in which this bacterium is first encountered.

Contributions of Fever to Tissue Repair

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Tissue repair is a complex biological process accomplished via intricate interactions between immune and connective tissue cells. Inflammation is critical for tissue repair since it is responsible for pathogen elimination and regulation of the subsequent tissue repair machinery. Our lab has established a crucial role of behavioural fever in the induction and resolution of inflammation. This study aims to investigate the contributions of fever to tissue repair. *In vivo* cutaneous infection of goldfish with *Aeromonas veronii* (Gram-negative bacteria) was induced, then fish were assigned to three distinct temperature categories: 1) fixed 16◦C; 2) fixed 26◦C, and 3) Dynamic (fever) temperature (allowing the fish to swim freely through a stable thermal gradient) for 14 days. Video monitoring of infected fish added to the thermal gradient showed a distinct fish preference towards higher temperature (dynamic/fever group). Fever was associated with early expression of pro-inflammatory cytokines and leukocyte recruitment followed by a rapid inflammation resolution. Wound closure was faster in dynamic compared to 16◦C. Quantitative PCR analyses revealed significantly higher expression levels of tissue repair genes under fever conditions. Although 26◦C fixed temperature showed some advantages relative to the 16◦C, it did not recapitulate all tissue repair benefits induced by fever, such as the maturation of epidermis and dermis layers of the skin. In conclusion, we propose that fever has a role in the restoration of homeostasis following injury/infection via achieving an efficient immune response as well as activation of tissue repair machinery. A mechanical increase in temperature offers some benefits but only partially recaps those offered through dynamic thermoregulation.

Novel use of long dsRNAi as an antiviral therapy across vertebrate species.

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To counter their continual exposure to viruses, vertebrates have evolved effective innate antiviral immune responses that are based on the type I interferon (IFN) system. Long double-stranded RNA (dsRNA), is a potent inducer of these IFNs and is produced by almost all viruses during replication. The intensity of the dsRNA-induced IFN response is dependent on dsRNA length, but not sequence. Another dsRNA-mediated antiviral response is the RNAi pathway, which induces viral knockdown in a sequence specific manner. Though intact and functioning in vertebrate cells, the role of RNAi as an antiviral response using long dsRNA (dsRNAi) remains controversial. The results presented here provide evidence that dsRNAi is not only functional, but has strong antiviral effects in rainbow trout gonadal cells (RTG-2) towards Chum Salmon Reovirus (CSV) when cells are pre-soaked with concentrations of sequence-specific dsRNA that are too low to induce IFN. This phenomenon was also observed in IFN-competent human fibroblasts (THF), human lung cells (MRC5) and human cancer cells (glioblastoma, SNB75) towards the rhabdovirus vesicular stomatitis virus expressing green fluorescent protein (VSV-GFP) and the human coronavirus, HCoV-229E. When THF and SNB75 were pre-soaked with GFP sequence-specific long dsRNA, a significant reduction in the TCID50 of VSV-GFP was observed when compared to mismatched sequence controls. When both cell lines were pre-soaked with dsRNA of VSV viral gene sequences (N and M protein), significant reductions in viral titres were detected. To confirm that this was not only a phenomenon with VSV-GFP, HCoV-229E was also examined. MRC5 were pre-soaked with viral gene dsRNA followed by viral infection. Though RdRp dsRNA was not protective, N, M and S protein dsRNA significantly reduced HCoV-229E titres. These results provide the first evidence that soaking with gene-specific dsRNA is capable of generating viral knockdown in vertebrate cells and is currently an untapped platform for antiviral therapies.

Session 3: Fish Innate immunity

Regional immune responses in the central nervous system of rainbow trout to systemic commensal bacteria

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Abstract: Extensive research studies have revealed how microbiota is crucial to maintain central nervous system (CNS) homeostasis. Imbalanced microbiome and translocation of commensals into the blood stream occurs in a number of disease states, several of which are associated with neurological manifestations. We hypothesized that regional differences to systemic bacteria exist in the CNS. Specifically, we propose that the olfactory bulb (OB) is a more immune effector region compared to the telencephalon (TL). To answer this question, we used rainbow trout as a model. Trout were injected intravenously with live commensal bacteria obtained from healthy trout skin and gut to mimic sepsis induced bacterial translocation. Four hours later, bacteria translocation into the OB, TL and spleen was measured by 16S rRNA gene qPCR and FISH staining. Results suggest higher presence of bacteria in the TL compared to the OB or the spleen. Microbiome sequencing revealed a selective entry of anaerobic gammaproteobacteria taxa into the TL with predicted functions in polyamine synthesis and catecholamine degradation. Higher basal expression levels of inflammatory cytokines (except for IL6), IgM, C3Ra and VCAM1 was detected in the OB compared to the TL, supporting its more immune effector role. In response to commensal i.v injection, the TL responses by up-regulating IL1b, TNFa and VCAM1 expression, whereas no changes were observed in the OB. Our findings reveal regional variability in the CNS in response to systemic commensal bacteria and suggest that bacterial invasion of the TL may lead to behavioral and neurological changes such as those observed in sepsis patients.

Boosting tilapia (*Oreochromis niloticus*) immune responses to *Flavobacterium columnare* using PACAP (pituitary adenylate cyclase activating polypeptide)

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Abstract:
Antimicrobial peptides have been isolated from variety of organisms and play an essential role in defense against infections. They are small peptides (below 60 amino acids) with broad spectrum antibacterial activity. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional neuropeptide that is capable of performing roles as a neuromodulator and vasodilator. In teleosts, PACAP has been demonstrated to have direct antimicrobial activity against several aquatic pathogens, including those from the genus Flavobacterium. Our goal was to examine the in vivo immunostimulatory capabilities of PACAP in tilapia and whether this was impacted by administration route. Over the course of two studies, tilapia(416.06+-116.66 g) randomly assigned to replicate tanks were administered either PACAP-38 or a modified form of PACAP-38, via intraperitoneal (i.p.)injection, bath, nares flush, or gill flush, and compared to PBS controls. Following individual treatments, tilapia underwent a bath exposure (40 L tank for 45 min) to *F. columnare* (isolate ALG-00-530; at 2.1×10^8 CFU/ml). or sham exposure without the addition of the bacterial culture. Fish were sampled prior to exposure, and 48 h after stimulation, at 1 day after the onset of mortality in exposed tanks, and at resolution of mortality. Spleen, skin, gill and nares samples were collected for quantitative gene expression and histological analysis. Tilapia that received i.p. injection of PACAP-38 showed significantly lower mortality (10%) than those receiving PBS i.p. (25%) from F. columnare. However, bath immersion of fish both with and without *F. columnare* resulted in significant mortality due to secondary infections with *Edwardsiella tarda*. Modified PACAP-38 however, initiated greater mortality in nares/gill flushed or i.p. injected fish compared to sham/PBS suggesting different forms of PACAP-38 are inducing different mucosal/systemic responses in tilapia.

The effects of climate change on the Atlantic salmon's (*Salmo salar*) immunological response to infectious salmon anemia (ISAv).

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Abstract:
Infectious salmon anemia is a reportable disease in Canadian aquaculture that severely affects the health of Atlantic salmon and results in millions of dollars in economic losses each year. With ocean temperatures continuing to rise due to global climate change, the industry has to discover ways to adapt and deal with the impacts the changing environment can have on immunological robustness of cultured fish. In this study, approximately 120 fish from each of 20 full or half-sib families were divided and cohoused in 38 different tanks. Half of the tanks were then acclimatized and maintained at 10°C (95-100% oxygen saturation), while the other half were acclimatized and maintained at 20°C (and 80-85% oxygen saturation). Donor Atlantic salmon were IP-injected with an ISAv isolate (HPR4) with a virulence of TCID50 of $1x10^5$/ml. One week after the injection, 6 donor fish were added to each study tank (ratio of 1:7, donor to naïve cohabitants). Mortalities were observed over the course of the study and percent mortality was calculated. A difference in survivorship was seen across the different temperatures and families. The three families with the highest mortalities (most susceptible), and the three families with the lowest mortalities (most resistant) were assessed for their antiviral responses using relative immune gene expression. Differences in anti-viral responses will be discussed with respect to relative resistance/susceptibility across family and the impact on temperature on these responses.

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DsRNA Formulated with Phytoglycogen Nanoparticles to Induce Innate Immune Responses in Rainbow Trout and Prevent Viral Replication of ISAV in Atlantic Salmon

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Abstract: Rainbow trout and Atlantic salmon are both economically important farmed aquatic species globally that are a source of nutrients to our ever-growing population. These farmed salmonid species rely heavily on their innate immune responses to defend themselves against invading pathogens. For this reason, using a prophylactic inducer of innate immune responses, such as double stranded (ds)RNA may be beneficial to prevent viral infections and mortality. Polyinosinic:polycytidylic acid (polyI:C) is a dsRNA molecule commonly used to stimulate innate immune responses. PolyI:C is a toll-like receptor (TLR) 3 agonist that induces the expression of type I interferons (IFNs) and interferon stimulated genes (ISGs) to create an antiviral state and prevent viral replication. Although polyI:C is an exceptional inducer of innate immune responses *in vitro* its usage *in vivo* has been limited due to instability. Thus, a phytoglycogen nanoparticle (NDx) was used in the present study to deliver the poly I:C. It was hypothesized that polyI:C when bound to NDx would induce stronger innate immune responses both locally and systemically compared to polyI:C alone in rainbow trout and Atlantic salmon, as well as prevent viral replication of an economically important aquatic pathogen, infectious salmon anemia virus (ISAV), in Atlantic salmon. Rainbow trout that received an oral gavage or feed pellets containing polyI:C-NDx had higher levels of expression of IFN1 and ISGs (Mx1 and Vig3) in the intestine and head kidney compared to rainbow trout that received polyI:C only. Similarly Atlantic salmon also expressed higher levels of expression of IRF7, Mx1 and Vig3, locally in the intestine and systemically in the head kidney as well as a reduced viral load of ISAV in the intestine, in comparison to the control group. The results of this study may help lead to new advancements of therapeutic drugs in the aquaculture industry to help prevent pathogenic outbreaks.

Pituitary adenylate cyclase-activating polypeptide (PACAP) as a prophylactic alternative against bacterial infections in rainbow trout (*Oncorhynchus mykiss*).

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Diseases caused by pathogens generate economic losses in aquaculture, and the widespread use of antibiotics has contributed to the development of antibiotic resistance, issue of concern for its repercussions on the production system, the environment and human health. Antimicrobial peptides (AMPs) constitute a promising strategy to develop new drugs for prevention and treatment of diseases, due to its fast and efficient response against pathogens. Pituitary adenylate cyclase-activating polypeptide (PACAP) have shown to have a strong antimicrobial activity and to play a role as a regulator of the teleost fish immune system. In this study, the synthetic *Clarias gariepinus* PACAP-38 (PACAP 1) and four PACAP-38 sequence variants (PACAP 2-5) were tested for cytotoxicity in a hemolytic assay using erythrocytes from three salmonid species, and in a cell viability assay using two rainbow trout cell lines, the monocyte/macrophage cell line from spleen, RTS11, and the epithelial cell line from gill, RTgill-W1. The results showed that only 50 µM PACAP 4 caused a hemolysis percentage higher than 20%, and none of the peptides at concentrations between 0.02 and 0.1 µM reduced the cell viability of the cell lines to less than 85%. The Minimum Inhibitory Concentration (MIC) of the peptides against six bacteria species including *Flavobacterium psychrophilum* was also studied. *In vitro* trials with RTS11 and RTgill-W1 exposed to *F. psychrophilum* showed that a 24-hour pre-treatment with 0.1 µM of different PACAP variants inhibited bacterial growth and modulated the expression of pro-inflammatory cytokines genes in both cell lines.

Developing reagents for assessing protein-level changes for cellular and oxidative stress responses in cultured Atlantic salmon (*Salmo salar*) in the face of temperature and hypoxia challenge.

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ABSTRACT
Climate change-related challenges, including increasing water temperatures and hypoxic events, threaten the sustainability of aquaculture in Canada. Developing quantitative protein-level assays for relevant stress and health biomarkers is critical for selecting Atlantic salmon (*Salmo salar*) families that are more capable of adapting to a rapidly changing abiotic environment, while maintaining robust immune responses. Salmon hepatic transcriptional responses to incremental increases in water temperature (maximum 20℃), in combination with moderate hypoxia (70% air saturation), were assessed using 44 k microarrays and validated via quantitative polymerase chain reaction. *Serpinh1* (encodes Serpin H1) transcripts were significantly upregulated relative to controls and targeted as a marker for heat shock response. Similarly, significant downregulation of *prdx6* (encodes peroxiredoxin 6) and *cirbp* (encodes cold-inducible RNA-binding protein) transcripts suggested that these genes were also relevant markers for cellular/oxidative stress and transcriptional regulation, respectively. With the aim of developing a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) for each of these candidate stress markers, recombinant proteins targets were produced in an *Escherichia coli* expression system using pET SUMO expression plasmids. Recombinant proteins were purified via immobilized metal affinity chromatography (IMAC), and further purified via size exclusion chromatography to generate mono-disperse protein samples free of contaminating bacterial proteins. Chickens and rabbits were immunized with these purified proteins to generate stress marker-specific polyclonal antibodies. Herein, details of protein production, refolding, and purification will be discussed, with special attention paid to the collaborative solutions used to overcome persistent challenges that have previously hindered the generation of high-affinity antibodies for use in quantitative ELISA assays. Rigorous execution of these protein production techniques is crucial for widespread and replicable use of quantitative ELISA assays by the research community at large, but especially for the accurate selection of salmon families likely to be least impacted by the effects of climate change.

Examination of a New Family of Fish Immune-Type Receptors may Reveal Novel Insights into the Mechanisms Controlling Vertebrate Innate Immunity

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Abstract: Innate immunity represents an important first line of host defense required for protecting an organism from pathogens. Generally, innate immune cells provide protection through various antimicrobial functions designed to kill microbial invaders. These responses are activated by specialized immunoregulatory receptor proteins on the surface of immune cells.

Immunoregulatory receptors transmit signals across the cellular membranes via cytoplasmic tail (CYT) regions, equipped with motifs which activate intracellular signaling cascades triggering potent responses such as phagocytosis (i.e. cellular engulfment and destruction of pathogens).

Our lab studies fish Leukocyte Immune Type Receptors (LITRs) as a model for understanding the conserved and divergent aspects of immunoregulatory receptor networks. Extensive work has already been performed on catfish LITR-types, revealing their ability to regulate innate responses. Recently, identification of LITRs in zebrafish has shown that these immune proteins contain novel arrangements of signaling motifs within their CYT regions but the significance of these new motifs for controlling immune responses has yet to be established. Overall, the focus of my research project is to characterize zebrafish LITR-types using phagocytosis-based immunoassays developed in our lab.

Based on the unique arrangements of the intracellular motifs and the predicted structure of the receptors, I hypothesis that zebrafish LITR-types employ versatile mechanisms of control over effector responses allowing for receptor based functional plasticity.

Work done so far has suggested that these new LITR-types can induce a phagocytic response when engaged with receptor specific targets that is dependent on classically defined activation motifs within the CYT. However, unique arrangements of signaling motifs also suggests that this response may be only part of the possible variable functions induced by zebrafish LITRs. Future testing will examine the possible versatility of immunoregulatory roles of selected LITR-types. Overall, characterization of the novel arrangements of motifs found within these fish immune proteins may lead to new understanding of the signaling mechanisms controlling vertebrate innate immunity.

Session 4: Amphibian innate Immunity

Amphibian Phagocyte Functions are Inhibited by the Deadly Chytrid Fungus, *Batrachochytrium dendrobatidis*

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Abstract: Chytridiomycosis, caused by the chytrid fungsus, *Batrachochytrium dendrobatidis* (*Bd*) continues to lead to amphibian population declines. Previously, we showed that *Bd* sporangia and supernatant factors inhibit amphibian and human lymphocytes and induce apoptosis. Here, we present evidence that the inhibition extends to phagocytic cells. Peritoneal phagocytes (enriched for MΦs and neutrophils) were induced by injection of killed bacteria into *Xenopus laevis* adult frogs and exposed to *Bd* sporangia or cell-free supernatant factors (*Bd* Sup) for 24 hr. Following the cell co-culture, fungal cells were removed by treatment with amphotericin B and a wash step to remove the antifungal drug. Treated phagocytes or untreated control cells were then exposed to pHrodo Green™ Zymosan Bioparticles™, and fluorescence of beads in the phagolysosomal compartments was measured after 2 hr. Phagocytes co-cultured with *Bd* Sup engulfed about 58.5 ± 9.9% fewer zymosan particles than untreated control cells (N=7 replicate experiments) while those directly exposed to *Bd* sporangia engulfed 61.4 ± 9.5% fewer zymosan beads that control cells (N = 3 replicate experiments) demonstrating that *Bd* cells can also inhibit this important cell function. Overall, these finding suggest another mechanism by which the fungal cells can evade immune clearance in the skin (Support NSF IOS-1557634 and IOS-2011291).

microRNAs and the regulation of innate antiviral immunity in frogs

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Abstract: microRNAs are key regulators of innate antiviral immunity in vertebrates, yet antiviral microRNAs remain uncharacterized in frogs. Frog skin represents the first line of pathogen defense, therefore we sought to profile the changes in microRNA expression in a *Xenopus laevis* (African clawed frog) skin epithelial-like cell line (Xela DS2) in response to Frog virus 3 (FV3) or poly(I:C) (a synthetic analogue of viral double-stranded RNA). Small RNA sequencing was performed on Xela DS2 cells that were untreated, infected with FV3, or treated with poly(I:C). We detected differential expression of 80 *X. laevis* microRNAs in response to FV3 infection or poly(I:C) treatment compared to controls. Target prediction analyses revealed that many of these microRNAs target important regulators of innate antiviral immunity, and gene ontology analysis of the predicted targets revealed the enrichment of terms such as "cell surface receptor signalling pathway", "response to cytokine", "intracellular signal transduction", and "response to hydroperoxide". Our study is the first to suggest that amphibian microRNAs play important roles in the regulation of innate antiviral immunity.

Just a gut feeling: Amphibian (*Xenopus laevis*) tadpoles and adult frogs mount distinct responses to intestinal Frog Virus 3 infections

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Abstract: Diverse amphibious species face rapid declines that are largely attributable to emerging pathogens such as the intracellular ranavirus, Frog Virus 3 (FV3). Most FV3 hosts are partly or fully aquatic; thus, waterborne viral transmission and infection often occur via the gastrointestinal tract. Host intestinal hemorrhages and necrosis are common in FV3 infections, which are more likely fatal in amphibian tadpoles than in adults. Yet despite this contribution to viral pathogenesis, life stage differences in amphibian immune responses to intestinal ranavirus infections remain poorly defined. As interferon (IFN) cytokines represent a cornerstone of vertebrate antiviral immunity, it is pertinent that the anuran *Xenopus laevis* tadpoles and adult frogs mount disparate IFN responses to FV3 infections. Here, we compare the tadpole and adult *X. laevis* immune responses to intestinal FV3 infections. We show FV3-challenged tadpoles mount more robust intestinal type I and III IFN responses than adult frogs. These tadpole antiviral responses are likely mediated by myeloid-lineage cells, which are recruited into the intestine in response to FV3 infection. Myeloid-lineage cells bearing similar cytology, however, already reside within the intestines of healthy (*i.e.*, uninfected) adult frogs, possibly accounting for some of their FV3 resistance. The present work provides critical insight into this host-pathogen relationship needed to address global amphibian declines.

The roles of amphibian (*Xenopus leavis*) macrophage subsets during chronic Frog Virus 3 infections

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Abstract: Infections by ranaviruses such as Frog Virus 3 (FV3) are significantly contributing to the global amphibian population declines. The anuran amphibian *Xenopus laevis* is an ideal animal model to study amphibian anti-ranaviral defenses and the roles of distinct frog leukocyte populations during FV3 infections. Frog macrophages (Mϕs) are thought to be integrally involved during FV3 infection as they serve as a means of viral dissemination/persistence but also participate in immune defense against this pathogen. In turn, Mϕ differentiation and functionality depend on the colony-stimulating factor-1 receptor (CSF-1R), which is ligated by CSF-1 and iterleukin-34 (IL-34) cytokines. Our past work indicates that *X. laevis* CSF-1 and IL-34 give rise to morphologically and functionally distinct frog Mϕ subsets and that these CSF-1- and IL-34-Mϕs respectively confer susceptibility and antiviral resistance to FV3. Because FV3 targets the frog kidneys and establishes chronic infections therein, presently we examined the roles of the frog CSF-1- and IL-34-Mϕs in seeding and maintaining these chronic kidney infections. Our findings indicate that the frog CSF-1-Mϕs result in more prominent kidney FV3 infections, which develop into greater reservoirs of lingering FV3. Conversely, the frog IL-34-Mϕs reduce animal FV3 loads and diminish the magnitudes of persisting virus. These disparate roles of CSF-1- and IL-34-Mϕs are marked by differences in hallmark immune gene expression within the kidneys of the respective Mϕ subset-enriched and FV3-infected frogs. Further studies of the mechanisms driving amphibian Mϕ susceptibility and resistance to ranaviruses will be invaluable to the development of remediation measures against these pathogens.

A novel *Xenopus laevis* model to evaluate the pathogenicity dissemination, and resistance of *Mycobacterium abscessus.*

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Mycobacterium abscessus (*Mab*) is a non-tuberculosis mycobacterium (NTM) responsible for pulmonary, cutaneous and disseminated infections in humans. *Mab* is abundant in nature and displays two colony morphotypes, smooth (S) and rough (R) depending on glycopeptidolipid (GPL) production. Despite some well-established features of the two morphotypes, we are still lacking knowledge of virulence mechanisms. The murine and zebrafish models have limitations due to the early clearance of *Mab* from mice and lack of lung and adaptive immunity of zebrafish embryos. *Xenopus laevis* tadpoles have both functional lungs and adaptive immunity that could contribute to unravel *Mab's* pathogenicity. We have already established a *Mycobacterium marinum* (*Mm*) model in *Xenopus* that we have extended to *Mab*. *Xenopus* tadpoles were inoculated with R- and S-*Mab* intraperitoneally and intracardially and organs were harvested at several time points (3 to 14 days) for profiling immune gene responses by qPCR and determining *Mab* dissemination by colony assay and fluorescence microscopy. Our data show that *S Mab* inoculated tadpoles had lower survival ratio compared to *R Mab* counterparts. Furthermore, transcript levels of inflammatory cytokines IL-1β, TNFα, and IFN-γ were increased in liver and spleen of *S Mab* inoculated tadpoles after 3 and 6 days, whereas *R Mab* infected organs showed iNOS gene upregulation after 14 days. These data suggest that *S Mab* induces acute immune response, which results in systemic inflammation and increased tadpole morbidity, whereas *R Mab* appears to trigger a delayed response accompanied by expanded tadpole survival. The exact mechanism of *R Mab* persistence and tadpole survival remains to be further investigated.

Thyroid Disrupting Chemicals Perturb Thymocyte Development in *Xenopus laevis*

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Abstract: Thyroid disrupting chemicals are ubiquitous water contaminants that cause varied health effects on humans and wildlife. Dysregulation of thyroid hormone signaling during perinatal development can weaken T cell function in maturity, raising the question of whether thyroid disrupting chemicals can perturb thymocyte development acutely. Using *Xenopus laevis* tadpoles as an exploratory model organism, we determined thyroid disrupting effects and thymocyte alterations following exposure to a mixture of thyroid disrupting chemicals used in unconventional oil and gas extraction (UOG) at concentrations well below those found in contaminated water. Notably, exposure to mixture chemicals reduced the number of CD8+ single positive thymocytes as well as double positive thymocytes, and co-exposure to mixture and exogenous thyroid hormones nullified the effect. These results suggest that thyroid disrupting chemicals can perturb thymocyte development through the thyroid hormone pathway. Pre-metamorphic exposure to the mixture chemicals delayed the timing of metamorphosis, and post-metamorphic froglets displayed defects in CD8+ thymocytes and splenic lymphocytes. Current experiments aim at determining whether exposure to thyroid disrupting chemicals during or just after the metamorphosis affect thymus morphogenetic changes and differentiation of adult T cells.

Session 5: MHC and Evolution

Histocompatibility response in Planaria and its mediation by cellular immune activity

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Abstract: Histocompatibility and allogeneic responses in mammalians are attributed to cytotoxic T cells and NK cells. Recent progress has revealed the presence of such responses in some colonial marine invertebrates; however, the mechanism through which immune rejections occur remains unclear as this response is mediated by species-specific mechanisms and may evolve independently. Here, by using diverse *in-vivo* and *ex-vivo* assays, ranging from cell isolation, cell and tissue transplantations, to genetic chimeras, we show the existence of histocompatibility and allogeneic cellular response in planarian flatworms. Together, our results suggest the cytotoxicity as means to modulate cellular rejection in planarians.

Major histocompatibility complex as an indicator of divergence in longnose gar

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Immunogenetic diversity between individuals within a population or species affects disease outcomes and drives adaptation. Characterizing this diversity allows us to better understand how the immune system evolves in different environments including pathogen exposure. The major histocompatibility complex (MHC) is one of the most diverse suites of genes in vertebrates. As a key player in the adaptive immune response, the MHC encodes proteins that process and present self and non-self peptide antigens on the cell surface. Specifically, MHC class I proteins possess a peptide binding region for binding and presenting antigens directly to immune receptors. In order for a species or population to present a wide range of non-self, pathogen-derived peptides, MHC class I genes are highly polymorphic, and serve as targets for selection. As a result, MHC class I genes often rapidly evolve in response to changing environments. Holostean fishes, gars and bowfin, are emerging genetic models for studying genome evolution. Holostei represent a lineage of ray-finned fishes that diverged from the teleosts, a diverse clade which includes over 26,000 species (e.g. carps, salmons, tetras, cods, catfishes and eels), around 300 MYA. In contrast to teleosts, holosteans are hypothesized to have changed very little over time and are a unique model to understand the evolutionary histories of both fishes and tetrapods. Using transcriptome data from longnose gar (*Lepisosteus osseus*) sampled from both Tennessee and North Carolina, we found that the MHC Class I *UBA* locus shows clear signatures divergence. We hypothesize that these signatures are indicative of either the radiation event facilitated by the Pleistocene glacial refugium, or by the geographic separation introduced by the Appalachian Mountains. The long-term goals of this project are to explore the coevolutionary patterns between MHC and interacting genes in two holostean species, longnose gar and bowfin.

Eight MHC class I lineages in the oldest vertebrates with human-like adaptive immunity

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Chondrichthyes (sharks, rays and chimaeras) is the most basal vertebrate taxon possessing the basic features of the adaptive immune systems based on MHC, and thus are a key group to understand the emergence and evolution of vertebrate adaptive immunity. Previous work has shown the presence of one classical (UAA) and two nonclassical (UBA and UCA) lineages of MHC class I genes in Chondrichthyans. In this study, we conducted in silico searches in all available Chondrichthyes databases and identified five new nonclassical MHC class I lineages (UDA, UEA, UFA, UGA and UHA) with completely different implied biochemical features and taxonomic distribution. UDA and UGA lineages are present in cartilaginous taxa but they are multicopy in Holocephali (chimaeras) and single/low copy in Elasmobranchii (sharks and rays). UFA and UHA are found so far only in Elasmobranchs and show striking differences in their structural features from all other lineages. UEA is a highly complex and multicopy lineage, found exclusively in sharks (Division Selachii). All of these new lineages, except UDA, lacks conservation of the nine invariant residues in the “peptide” (ligand)-binding regions (PBR) in most vertebrate classical class I proteins, and thus they are likely nonclassical-type. The PBR also range from hydrophobic (UEA, UCA, UFA) to hydrophilic (UDA) compared with the classical lineage UAA, suggesting that different lineages associate with different ligands. For the first time, we show that the Chondrichthyian fish already show a remarkable diversity of a key immune gene family, MHC class I, despite their ancient origin and position at the base of jawed vertebrates.

Cartilaginous fish class II genes reveal 350 My-old allelic lineages in sharks but no DM

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Abstract: Cartilaginous fish (chimaeras, rays and sharks) are the most basal extant jawed vertebrates with an adaptive immune system based on the Major Histocompatibility Complex (MHC). However, no comprehensive characterization of MHC class II genes has been undertaken for the group. We performed extensive bioinformatic searches on a taxonomically diverse dataset of transcriptomes and genomes of cartilaginous fish targeting MHC class II sequences. Class IIα and IIβ sequences were retrieved from all taxa analyzed and showed typical features of classical class II genes. Phylogenetic analyses of the immunoglobulin superfamily domain showed two divergent, ancient lineages of class II genes across different shark Orders, originating >350 million years ago in the late Devonian. Linkage of lineage-specific pairs of IIα and IIβ genes was found, with genes segregating as distinct biallelic lineages. Ongoing work is currently assessing genetic diversity between class II lineages in sharks, both at the species- and population-levels, as well as testing for differential gene expression levels between lineages in eleven different nurse shark tissues. Nonclassical class II sequences (e.g. DM and DO) were not retrieved from these data, and classical class II sequences lacked the conserved residues shown to interact with DM molecules, supporting claims that the DM system arose only in the lobe-finned fish lineage leading to tetrapods.

Deciphering the role of MHC class I-like XNC4 interacting T cells during mycobacterial infection in *Xenopus*

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Abstract: Tuberculosis and non-tuberculosis mycobacteria are major health concern in human because of the lack of effective treatments. In fact, new therapeutic strategies have been limited by the lack of knowledge on the interaction between mycobacteria and the host. MHC class I-like restricted innate-like (i)T cells (CD1d-restricted iNKT and MR1-restricted MAIT) are involved in the early immune response against *Mycobacterium tuberculosis* in mammals. Nevertheless, their role remains to be fully characterized. For the first time in a non-mammalian species, iT cells have been characterized in the amphibian *Xenopus*. Notably, reverse genetic approaches have revealed that MHC class I-like XNC4-restricted iVα45 T cells expressing invariant Vα45-Jα1.14 TCR have a critical non-redundant role in tadpoles' resistance against *Mycobacterium marinum* (*Mm*). In contrast to adult frogs, tadpole is more tolerant to *Mm* and it immunity relies predominantly on iT cells. We, therefore, hypothese that XNC4 presenting cells and interacting iT cells are critical for immune tolerance of *Xenopus* tadpoles against *Mm*. Preliminary results indicate that XNC4 binds unusually long peptides (9-14 mers). Using a combination of XNC4 specific nanobody (Nb5), XNC4-β2m tetramer (XNC4-T), fluorescent DsRed+ *Mm,* and recombinant proteins including CSF1, we characterized by flow cytometry the recruitment of putative XNC4 presenting cells and XNC4-restricted T cells in tadpoles and adults frogs after intraperitoneal *Mm* injection. Our study confirms that tadpole's immune system in more tolerogenic: tadpoles myeloid cells show higher DsRed+ *Mm* load despite a CD8 T cell immune reaction detected for the first time in tadpoles. In addition to the rectruitement of XNC4-restricted T cells, recruited Nb5+ cells consit in heterogenous immune cell populations including lymphocytes, macrophages and neutrophils showing different recruitment kinetics in adult and tadpoles. Importantly, Nb5+ macrophage show a dominant M2-like phenotype, which reinforce our working hypothesis and provide new insight into the role of iT cells during mycobacterial infection.

Cell surface expression of the non-polymorphic MHC class I-like molecule XNC4 does not strictly require b2-microglobulin association

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Evolutionary conservation of the innate and adaptive immune systems in multiple species can inform us about conserved host-pathogen interactions. Unlike classical MHC class I (class Ia) molecules, MHC class I-like (class Ib) molecules may not strictly require beta 2-microglobulin (*b2m*) and peptide association for surface translocation (e.g., HLA-F). XNC4 is a non-polymorphic class Ib in the amphibian *Xenopus laevis* that confers resistance against the non-TB *M. marinum* (*Mm*). *Mm* serves a model organism to study *Mtb*. *Xenopus* tadpoles are ideally suited to investigate class Ib function because they are naturally class Ia-deficient and have prominent class Ib-interacting innate-like T cell populations and functional lungs. Based on preliminary data that suggests that XNC4 binds unusually long peptides (11-15mer) reminiscent of human HLA-F, we hypothesize that **XNC4 presents unusually long mycobacteria-derived peptides in a TAP- and *b2m*-independent manner**. Accordingly, we engineered recombinant tagged XNC4 and X. laevis b2m. Preliminary data indicate that similar to HLA-F, XNC4 is detected at the cell surface of transfected cells without requiring laevis b2m co-transfection and co-transfection with b2m doesn't markedly increase expression of XNC4. We are currently optimizing transfection methodology to examine in more detail possible b2m association to a fraction of XNC4 and requirement of peptide processing. Elucidating the mechanism of action of XNC4 may provide a better understanding of host-pathogen interactions and their conservation in comparison to human host infected with TB.

Olfactory receptors on lymphocytes in a marsupial

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Abstract: Olfactory receptors (OR) mediate the sense of smell in the olfactory bulb. Recently, ORs have been shown to be expressed on non-olfactory tissues including cells of the immune system. An analysis of single cell transcriptomes of opossum splenocytes and peripheral blood mononuclear cells, revealed that ORs were expressed on a subset of T cells, the γμT cells, which are only found in marsupials and monotremes. The ORs on γμT cells are members of the OR14 family that is also uniquely expanded in the genomes of marsupials and monotremes. These ORs were found on a majority of the γμ T cells (~61%) but were not well annotated. To identify specifically which OR14s are specifically present on γμT cells, we annotated this family in the opossum genome. Of the OR14s, the subfamily C (OR14C) was expressed on γμ T cells. OR14C has 21 members but only four (OR14C2, C3, C5, and C9) are found on γμT cells. Opossum OR14s are broadly expressed and found in brain, diaphragm, kidney, ovary, stomach, teste, and thymus. These studies provide the basis for future determination of the function of ORs on non-olfactory tissues.

Session 6: Ig superfamily receptor evolution

The third domain of T cells: Defining the uniquely mammalian γμ T cell

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Abstract: An important component of the adaptive immune system are T cells. T cells are divided into two distinct lineages, using either the αβ or γδ T cell receptors (TCRs), and both are found in nearly all jawed vertebrates. Here, we describe a T cell population, γμ T cells, only found in marsupials and monotremes. γμ T cells have a distinct TCR structure with a highly diverse, antibody-like V domain that is unusual in being unpaired, much like light-chainless antibodies. An analysis of single cell transcriptomes from unsorted adult opossum thymus, spleen and peripheral blood cells was performed to investigate the presence and phenotype of γμ T cells in this model marsupial. The following conclusions can be drawn: 1) γμ T cells were absent from both adult opossum thymus and peripheral blood; 2) γμ T cells represent 10% of splenocytes and 37% of splenic T cells; 3) 75.5% of splenic γμ T cells are CD8αα^{+} while none express CD4; and 4) γμ T cells have a transcriptome dissimilar from αβ T cells consistent with having a distinct function. Analyses of TCRμ and TCRγ transcripts using both RT-PCR and a publicly available opossum transcriptome database revealed limited tissue distribution of γμ T cells in adult opossums. γμ T cells appear limited to the spleen, liver, stomach, and colon. Collectively, these results confirm a unique and ancient third lineage of mammalian T cells.

Structural characterization of the marsupial γμ T cell receptor

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Abstract: Most T cells found in jawed vertebrates express functional heterodimeric receptors (TCRs) on their surface formed by either α and β or γ and δ chains. Each chain possesses two domains, an amino-terminal variable domain (V) and a constant domain (C) on the carboxy-terminus (V-C pattern). In most cases, the ability of T cells to recognize diverse antigens relies on the surface (or paratope) located within Vα – Vβ or Vγ – Vδ segments. Recent genomic studies of non-eutherian mammals identified clusters of genes that resemble the classical TCR loci but surprisingly contain an additional variable segment. The functional product common for marsupials and monotremes called 'μ chain' was predicted to contain two variable (Vμ and Vμj) and one constant (Cμ) domains. Single cells analysis of blood and spleen from *Monodelphis domestica* showed that some of the splenic T cells co-express the μ and γ chains suggesting that both polypeptides could form a novel type of T cell receptor, the γμTCR. Using obtained sequences, we generated and structurally characterized two different γμTCRs. Here, we present the novel and unusual architecture of a third lineage of T cell receptor found in marsupials and monotremes **[1]**.

[1] Morrissey K.A., Wegrecki M., Praveena T., Hansen V.L., Bu L., Sivaraman K.K., Darko S., Douek D.C., Rossjohn J.[#], Miller R.D.[#], Le Nours J.[#] The molecular assembly of the marsupial T cell receptor defines a third T cell lineage. *Science*, 371, 1383-1388, 2021.

A highly diverse set of novel immunoglobulin-like transcript (NILT) genes in zebrafish indicates a wide range of functions

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Abstract: Multiple novel immunoglobulin-like transcripts (NILTs) have been identified from salmon, trout and carp. NILTs typically encode activating or inhibitory transmembrane receptors with extracellular immunoglobulin (Ig) domains. Although predicted to provide some level of immune recognition in ray-finned fish, we currently lack a definitive framework of NILT diversity, thereby challenging our ability to understand the evolutionary origin and specific function of these genes. In order to better understand the diversity of NILT genes and their possible roles in immune function, we identified 5 NILT loci in the Atlantic salmon (Salmo salar) genome, defined 86 NILT Ig domains within a 3 Mbp region of zebrafish (Danio rerio) chromosome 1, and described 41 NILT Ig domains as part of an alternate haplotype for this same genomic region. We then identified transcripts that define 43 different NILT genes and reflect an unprecedented range of sequence diversity and combinatorial diversity of Ig domains for a single family of non-recombining receptors. Zebrafish NILTs include a single putative activating receptor but extensive inhibitory and secreted forms as well as membrane-bound forms with no known signaling motifs. Whole genome sequences from different genetic backgrounds reveal at least three alternative haplotypes of the zebrafish NILT gene cluster that display gene content variation. This observation indicates that different individual fish can encode different combinations of NILTs which could impact their immune function. Furthermore, these results reveal a higher level of genetic complexity and sequence diversity for NILTs than previously described, suggesting that this gene family likely plays multiple roles in host immunity.

Expression analyses of CaLITRs in response to various immune stimuli

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Leukocyte immune-type receptors (LITRs) are polymorphic and polygenic members of the immunoglobulin superfamily (IgSF) that were originally discovered in the channel catfish. Over the past decades, functional characterization of LITRs using heterologous overexpression systems (i.e., AD293 cells) has shown that select LITRs exhibit robust immunoregulatory potentials and signaling versatility. Recently, various LITR cDNAs have been identified from goldfish primary kidney macrophages (PKMs) and neutrophils (PKNs). Sequence analyses of the various *Carassius auratus* L. (Ca)-LITR transcripts have revealed that they have variable number/arrangements of Ig-like domains and signaling potentials. Subsequent genomic analyses of the CaLITR cDNAs have found that they are located on various goldfish chromosomes (Chr). For example, CaLITR2, a predicted activating receptor-type with an ITAM-like domain containing tail, is located on Chr47. CaLITR3, a putative activating receptor-type with a positively charged histidine-containing transmembrane region, is located on Chr3. Lastly, CaLITR4, a receptor-type with diverse signaling potentials, is located on ChrLG28B. The different genomic locations of the various CaLITRs suggest that these genes may be under the regulation of different cis- or trans-regulatory elements and have variable expression patterns in response to immune stimuli. To gain more insights into how different immune-stimuli potentially influence the expression patterns of CaLITRs from variable genomic regions, we used a quantitative (q)-PCR-based approach to examine the expression profiles of representative CaLITRs (i.e., CaLITR1,2,3,4, and 5) located on different goldfish chromosomes under different immune-contexts (mixed leukocyte cultures (MLR) and following in vivo stimulation of goldfish with zymosan). Our results show that the various CaLITRs examined exhibited different expression patterns in both immune conditions. Specifically, CaLITR1 is significantly upregulated over 48 hours in MLR, whereas no significant upregulation of CaLITR2,3, 4, and 5 was observed under the same condition. Conversely, CaLITR1 showed no significant upregulation in peritoneal cells extracted from goldfish injected with Zymosan. Comparatively, CaLITR2 was highly expressed at 24 hours post-zymosan injection, whereas CaLITR3,4, and 5 were significantly upregulated in peritoneal cells 48 hours post-zymosan injection. Overall, these results have shown that CaLITRs have variable expression profiles in different immune contexts and provide the basis for further investigation of the functional significance of the upregulation of CaLITRs in these inflammatory settings.

The evolutionary history of receptors for immunoglobulins reveals the origin and the complexity of adaptive immune systems at the base of tetrapod evolution

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Abstract: Through coevolution with microbes, fishes developed and refined an adaptive immune system to match and anticipate the diversity of potential pathogens. Expansion of the B lymphocyte compartment and the antibodies they produce are correlates of infection. However, nothing is known about the functional consequences, the relevance, and the implications of these changes for fish health, because the leukocyte populations and molecules interacting with antibodies are poorly studied. Since these cells and receptors are evolutionarily and intimately associated with immunoglobulins (Igs), we have performed phylogenetic analyses on putative Ig receptors to find likely homologs of Ig receptors known in higher vertebrates. To this end we performed large-scale genome searches in cartilaginous (Chondrichthyes) and bony (Osteichthyes) fishes to elucidate the evolutionary history of Ig receptors in these ancestral groups of vertebrates. Using an extensive database of vertebrate homologs, we identified orthologs of polymeric-Ig receptors (pIgRs) and fragment crystallizable (Fc) receptors in cartilaginous and bony fishes. Phylogenetic analyses supported a massive expansion of pIgRs in bony fishes but not in cartilaginous fish with the exception of skates. Notably the phylogeny of both Fc receptors and pIgRs receptors revealed a putative origin of mammalian FcαμR and Fcα in bony fishes. Our analyses also revealed that chondrichthyes represent the first organisms to possess true Igs along with Ig receptors. Despite cartilaginous fishes being the first organisms to possess true immunoglobulins, and the concurrent expansion of Ig receptors, the functional significance of these receptors remains enigmatic. Strikingly, our resuls suggest unprecendented heterogeneity of Fc, Fc-like, and pIg receptors disproportionate to the limited number of isotypes in fish relative to mammals, and suggests a higher level of regulation and complexity of the two major Ig classes produced by bony fishes.

Expression of putative Fc receptors in different immune cell types of the rainbow trout (*Oncorhynchus mykiss*)

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Abstract: In all vertebrates including teleost fishes, adaptive and innate immune responses work synergistically to protect the host. One key component interacting with both members of the innate and adaptive immune systems is the antibody, which can target a variety of antigens owing to the high variability of the antigen receptors. An important part of antibody-antigen complexes is the fragment crystallizable (Fc) region of the immunoglobulin molecule. In mammals, receptors with affinity for these regions (Fc receptors) are found on certain cells of the immune system including B cells, myeloid cells, mast cells and others, and enable antibody-dependent effector functions. However, in bony fishes their existence and the heterogeneity of their expression on various cell types as well as in different tissues remains unknown. In this study, we aimed to fill the gap in the available knowledge and elucidate the expression of selected Fc receptors in different tissues and cell types of the rainbow trout. To this end, we have evaluated the expression of FC receptors in the main systemic and mucosal immune organs, as well as in individual subpopulation of blood cells, including the MACS sorted myeloid cells, IgM+ B lymphocytes, thrombocytes and erythrocytes. The obtained data provided new insights on the possible involvement of studied Fc receptors in variety of tissues including spleen, head kidney, trunk kidney, liver, gills, intestine, adipose tissue, muscle and heart. Furthermore, we have observed high linieage specificity of FC receptor expression in sorted populations of leukocytes. Unexpectedly, our data also identified unprecendented expression of FC receptors on erythrocytes, suggesting their potential role in the FC receptor mediated binging of Igs. Overall, the lineage specificity of Fc receptor expression highlights their specialized roles in the immune system of teleost fishes and allows future studies of their effector roles on individual leukocytes substes.

Session 7: B cell function and evolution

Evolutionary Precursors of Germinal Centers are Present in Sharks

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Abstract: Sharks are members of the oldest extant taxonomic group (Chondrichthyes) to have immunoglobulin (Ig)-based adaptive immunity. While previous work has shown sharks can affinity mature their B cell repertoires it is reported that they lack the specialized germinal centers (GCs) that mediate this process in mammals. Thus, we hypothesized that sharks possess structures that are the evolutionary foundation of GCs in mammals. To identify these sites, nurse sharks (*Ginglymostoma cirratum*) were immunized with the fluorescent antigen phycoerythrin (PE) to visualize antigen trafficking *in situ*. Spleen samples were collected and analyzed using immunofluorescent microscopy (IF) and RNA fluorescent in situ hybridization (RNA FISH). IF experiments showed antigen is clustered in the center of Ig-positive B cell follicles at 40 days post-immunization. RNA FISH experiments demonstrated aggregates of T cells at the borders of B cell follicles and putative T follicular helper cells within the follicles. AID expression appears to be upregulated in IgNAR+ B cells at the periphery of the follicles. Finally, a chemokine gradient of CXCR4/CXCR5 expression appears to direct cell migration, akin to the light zone and dark zone demarcation in mammalian GCs, with CXCR4 expression overlapping regions of AID expression. We believe we have identified primordial sites of B cell selection in the nurse shark spleen and that these are the evolutionary precursors to canonical GCs. Future work will focus on determining how this model influences the B cell repertoire.

Ab-normal erythrocytes in proliferative kidney disease (PKD) – rainbow trout (*Oncorhynchus mykiss*) infected by *Tetracapsuloides bryosalmonae* harbor IgM$^+$ red blood cells

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Abstract: *Tetracapsuloides bryosalmonae* is the etiological agent of proliferative kidney disease (PKD) in salmonid fishes, notably the commercially farmed rainbow trout *Oncorhynchus mykiss*. Infected fish can be identified by swollen kidneys and anemia among other signs. Some of these external signs of PKD can be explained by cellular level changes such as B lymphocyte proliferation/hyperplasia in the kidneys, but the B cell response alone does not account for all the clinical signs and pathologies associated with PKD such as anemia and pale gills. While attempting to study B cells in a population of infected commercially farmed rainbow trout during the peak of seasonal PKD, we unexpectedly detected another cellular abnormality in the form of IgM$^+$ red blood cells (RBCs). We verified the presence of surface IgM via parallel approaches: flow cytometry, fluorescence microscopy, and identification of IgM from the surface fraction of the RBC proteome. The frequency of IgM$^+$ RBCs (shifting as a whole population) and the magnitude of the phenotype have not been described in homeostasis nor in other diseases of teleost fishes. The absence of self-reactive Igs in the plasma of PKD fish, the lack of hemolytic activity in this plasma, and the stability of the phenotype are evidence that the IgM is not bound to an antigen on the erythrocyte surface, but is instead captured by an unidentified Ig receptor. Overall, we have identified an aberrant population of RBCs bearing a potential novel marker of PKD. This marker needs to be further studied and contextualized with what we know about the humoral response towards myxozoan parasites in order to determine if the IgM is as much a driver of pathology (via interaction with antigen and/or a receptor) as it is an indicator of disease.

Antibody affinity modification in fish

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Antibody affinity maturation is the process to improve antibody affinity for an antigen during a humoral immune response; it is mediated by the immunoglobulin (Ig) mutator enzyme activation-induced cytidine deaminase (Aicda). In homeotherms, this process occurs in distinct structures called germinal centers (GCs). Fish were thought to lack affinity maturation because they lack histologically distinct GCs. However, a functional homologue of Aicda has been identified in fish, where Aicda expressing cells co-exist with a population of pigmented cells called melano-macrophages (MMΦs). These aggregates are associated with the essential components of GCs. We hypothesized that these clusters are functionally analogous to GCs. To test our hypothesis, we generated whole VDJ repertoires for melano-macrophage clusters (MMΦCs) from vaccinated and unvaccinated zebrafish. Our results have shown that each cluster is dominated by up to four clonally expanding B-cells. In some clusters, 2-3 activated cells proliferate and acquire mutations in addition to the dominant clones.

To determine if there is an active selection process within MMΦCs, we examined the distribution of replacement vs. silent mutations on CDRs and framework regions (FRs) within the variable domain of the Ig. CDRs are the sites that determine the Ag binding affinity while FRs provide the structural backbone of the Ig. Therefore, we generally expect to see a higher ratio of R to S mutations on CDRs but not on FRs if there is a mechanism for selection. Although the R/S ratio varied among the different repertoires, their CDRs had a higher R/S estimate compared to the FWRs. Also, using Hill numbers we found low clonal diversity and more related clones within MMΦCs, which could indicate the presence of an effective recruitment mechanism within these clusters, where few B-cells are recruited and diversified.

These results provide strong evidence of affinity modification occurring in organized cellular structures in fish much as GCs in mammals.

Single cell-RNA seq profiling of spleen cells in naïve and immunized threespine stickleback fish (*Gasterosteus aculeatus*)

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Abstract: Teleost spleen is rich in melanomacrophage centers (MMCs), histologically distinguishable aggregates of highly pigmented phagocytes. In addition to the storage of unmetabolized materials and erythrophagocytosis, MMCs also phagocytose infectious microorganisms such as bacteria and fungi. The observation of antigen retention on or in close proximity to MMCs, the expression of germinal center (GC) specific genes, along with the presence of B cells led to the conceptualization that these centers are evolutionary precursors to GCs, and thus contribute to the development of adaptive humoral immune response. To the end of creating an unbiased profile of the spleen cells and elaborating the cellar and molecular kinetics that underlie the innate and adaptive immune responses, single cell-RNA sequencing was applied to spleen cells isolated from naïve, alum (Adjuvant)-injected and NP-CGG in adjuvant-injected (Immunized) threespine stickleback fish, respectively. The preliminary results showed the presence of 12 distinct cell populations in the spleen, comprising major leucocyte types (e.g., B cells, antigen-presenting cells (APCs), and granulocytes), erythrocytes, and other cell types. The proportion of cell populations varied between the differently treated stickleback groups, with the high percentages being mainly observed in immunized fish. This study will provide a comprehensive atlas of the stickleback spleen at single-cell resolution, and a better understanding of the immune response kinetics in bony fish.

Guardian of the genome: the prequel to V(D)J recombination by RAGs

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Abstract

The appearance of adaptive immunity in jawed vertebrates is termed the immunological 'Big Bang' because of the short evolutionary time over which it developed. Underlying it is the RAG-based V(D)J recombination system, which creates the diversity of the immunoglobulins and lymphocyte antigen receptors. It was convincingly shown that the *RAG1/2* genes originated from a single transposon. The current dogma postulates that the V(D)J system was established by at least two genomic insertions of this transposon, leading to a gene that was split into *V(D)J* segments in parallel with the transposon domestication as the RAG recombinase. Here we propose an alternative evolution hypothesis suggesting that the RAG1/2 transposase was first domesticated to serve as a genome guardian against invasions of closely related transposons. As the genome guardian, early domesticated RAG may have eliminated all but one *Transib* invader with asymmetric TIRs, which survived because of the evolutionary advantage it provided in splitting the primordial vertebrate immune receptor into *VDJ* segments. This hypothesis offers a prequel to V(D)J recombination by RAGs

Proteasome subunit 8 (PSMB8) of ray-finned fishes; cataloging trans-species polymorphisms

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Abstract: Proteasome subunit 8 (PSMB8) is part of the inducible proteasome complex that is encoded in the major histocompatibility complex (MHC) and is responsible for systematically degrading proteins into short polypeptides for loading on and display by MHC proteins. The 31st amino acid of the mature PSMB8 protein is often found to be either an alanine or a phenylalanine and can be used to classify MHC haplotypes for a given species (PSMB8A or PSMB8F, respectively). However, investigations across multiple species such as zebrafish (*Danio rerio)*, Japanese smelt (*Hypomesus nipponensis*), and rainbow trout (*Oncorhynchus mykiss*) have identified both PSMB8A and PSMB8F MHC haplotypes. These trans-species polymorphisms may lead to functional changes in the sequences of proteins degraded by the proteasome and thus the antigens displayed. To determine the extent of PSMB8 trans-species polymorphism across ray-finned fishes, we developed a bioinformatic pipeline to investigate all available fish genomes on Ensembl and NCBI repositories. Our preliminary results suggest the number of trans-species polymorphisms at the PSMB8 gene in fishes is much greater than initially estimated. Moreover, we found that the PSMB8 gene in holostean species (e.g., bowfin and gar), encodes novel residues, threonine or serine, at the 31st position. Our results indicate that these PSMB8T/PSMB8S alleles are restricted to holosteans and do not occur in any other fish species. Collectively, our results have great potential for optimizing vaccine development for aquaculture species at the level of the individual.

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