NACIW 2016 schedule

At-a-glance:

Tues, June 21st		Wed June 22nd		Thurs June 23rd	
		8.00-9.00	Registration		
			Early evolutionary development of immunity		Comparative Immunology at work in Atlantic Canada
		9.00-9.05	Welcome 5 mins	9.00-9.40	Gagne
		9.05-9.35	Oren	9.40-10.00	Braceland
		9.35-10.05	Fugmann	10.00-10.20	Hori
		10.05-10.35	Criscitiello	10.20-10.40	Fast
		10.35-10.50	Discussion	10.40-11.00	Coffee
		10.50-11.10	Coffee		Development of Immunity
			Pathogens	11.00-11.20	Ferenkamp
		11.10-11.30	Whyte	11.20-11.40	Robert
		11.30-11.50	Semple	11.40-12.00	Lutton
		11.50-12.10	Carpio		
		12.10-12.30	Rollins-Smith	12.00-13.00	Lunch/Business meeting
		12.30-13.00	Lunch		
			Regulation and Macrophage		Genomics and Transcriptomics
		13.00-13.20	Rast	13.00-13.20	Buckley
		13.20-13.40	Sunyer	13.20-13.40	Schuh
		13.40-14.00	Schrankel	13.40-14.00	Clark
		14.00-14.20	Takizawa	14.00-14.20	Casadei
		14.20-14.40	Lee	14.20-14.40	Purcell
		14.40-15.00	Yoder	14.40-15.00	Deiss
		15.00-15.20	Coffee	15.00-15.20	Coffee
			Self Recognition: MHC, TCR and more		Innate immunity: Antiviral and PRRS
		15.20-15.40	Mortimer	15.20-15.40	Le Nours
		15.40-16.00	Braden	15.40-16.00	Monjo
		16.00-16.20	Banach	16.00-16.20	Smith
		16.20-16.40	Park	16.20-16.40	Lisser
		16.40-17.00	Heimroth	16.40-17.00	Roux
15.00-17.00	Registration	17.00-18.00	Posters	17.00-17.20	Poynter
				17.20-17.40	Pham
19.00-22.00	Opening Reception	19.00-??	Dinner on your own or Informal Lobster dinner	19.00-22.00	Banquet

Tuesday June 21st:

15.00-17.00 Registration: Atlantic Veterinary College, Learning commons

20.00-22.00 Opening Reception: Upstreet Brewery
(Registration will be available here)

Wednesday June 22nd: Lecture Hall A, Atlantic Veterinary College

8.00-9.00 Registration (In front of Lecture Hall A)

9.00-9.05	Welcome	
	Special session:	Early evolutionary development of adaptive immunity
9.05-9.35	Matan Oren	REDEFINING GENE DIVERSIFICATION DOGMAS: LESSONS FROM THE IMMUNE SYSTEM OF ECHINODERMS
9.35-10.05	Sebastian Fugmann	IDENTIFICATION OF NOVEL IMMUNE MEDIATORS IN THE TRANSCRIPTOME OF STRONGYLOCENTROTUS PURPURATUS COELOMOCYTES
10.05-10.35	Mike Criscitiello	SOMATIC HYPERMUTATION AND USE OF IMMUNOGLOBULIN VARIABLE REGIONS AT SHARK T CELL RECEPTORS
10.35-10.50	Discussion	
10.50-11.10	Coffee	
		Pathogens
11.10-11.30	Shona Whyte	VACCINE-INDUCED PROTECTION AGAINST BACTERIAL INFECTIONS IN ARCTIC CHARR (*SALVELINUS ALPINUS*)
11.30-11.50	Shawna Semple	USING OUTBREEDING TO IMPROVE THE IMMUNOLOGICAL PERFORMANCE OF CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTCHA*) IN RESPONSE TO VIBRIO ANGUILLARUM.
11.50-12.10	Yamilla Carpio	AN IMMUNOGENIC PEPTIDE DERIVED FROM THE RIBOSOMAL PROTEIN P0 AS VACCINE CANDIDATE FOR THE CONTROL OF SEA LICE INFESTATIONS
12.10-12.30	Louise Rollins-Smith	IMMUNOTOXICITY OF GEOGRAPHICALLY DIVERSE ISOLATES OF A PATHOGENIC AMPHIBIAN CHYTRID FUNGUS
12.30-13.00	Lunch	
		Regulation and Macrophages
13.00-13.20	Jonathan Rast	GENE REGULATORY NETWORK CONTROL OF IMMUNE SYSTEM DEVELOPMENT AND RESPONSE

13.20-13.40	Oriol Sunyer	FIRST IDENTIFICATION OF REGULATORY B CELL SUBSETS EXPRESSING IL-10 IN A NON-TETRAPOD SPECIES
13.40-14.00	Catherine Schrankel	A CONSERVED ALTERNATIVE FORM OF THE PURPLE SEA URCHIN HEB/E2-2/E2A TRANSCRIPTION FACTOR MEDIATES A SWITCH IN E-PROTEIN REGULATORY STATE IN DIFFERENTIATING IMMUNE CELLS
14.00-14.20	Fumio Takizawa	CHARACTERIZATION OF EVOLUTIONARILY CONSERVED CD4+ MONOCYTES/MACROPHAGES IN RAINBOW TROUT
14.20-14.40	Lucy Lee	EVALUATING PHAGOCYTIC ABILITY OF RTS11-GFP, A STABLY TRANSFECTED SUB-LINE OF RTS11, A MONOCYTE/MACROPHAGE CELL LINE FROM SPLEEN OF RAINBOW TROUT
14.40-15.00	Jeffrey Yoder	DISRUPTION OF TRIM9 FUNCTION ABROGATES MACROPHAGE MOTILITY *IN VIVO*
15.00-15.20	Coffee	
		Self Recognition: MHC, TCR and more
15.20-15.40	Nathan Mortimer	AUTOIMMUNITY AND IMMUNE RECOGNITION IN THE DROSOPHILA MELANOGASTER tuSz MUTANT
15.40-16.00	Laura Braden	RESOLUTION OF KUDOA THYRSITES INFECTION IS ASSOCIATED WITH INFILTRATION OF MHIIβ+ CELLS IN ATLANTIC SALMON, *SALMO SALAR*
16.00-16.20	Maureen Banach	REVERSE GENETIC ANALYSIS OF XENOPUS LAEVIS NONCLASSICAL MHC CLASS IB GENES BY CRISPR/CAS9-BASED GENOME EDITING.
16.20-16.40	Jules Park	DEVELOPING A MODEL IN XENOPUS TO STUDY NONCLASSICAL MHC IB-RESTRICTED INNATE T CELLS IN MACROPHAGE-MEDIATED ANTI-*MYCOBACTERIUM MARINUM* RESPONSES
16.40-17.00	Ryan Heimroth	CHARACTERIZATION OF T CELL RECEPTOR MOLECULES IN AFRICAN LUNGFISH (*PROTOPTERUS DOLLOI*)
17.00-18.00		Posters (Cash bar)
P1	Jules Park	TWO DIFFERENT PROMINENT NONCLASSICAL MHC CLASS I-RESTRICTED INVARIANT T CELL LINEAGES WITH NON-OVERLAPPING CRITICAL ANTIVIRAL AND ANTI-MYCOBACTERIAL IMMUNE FUNCTIONS IN THE AMPHIBIAN XENOPUS
P2	Ryan Schell	FOLLOWING THE HUMORAL IMMUNE RESPONSE IN NATIVE NEW ENGLAND AMPHIBIANS USING REAGENTS DEVELOPED IN *XENOPUS LAEVIS.*
P3	Melanie Gallant	DEVELOPMENTAL EXPRESSION PROFILES AND THYROIDAL REGULATION OF CYTOKINES DURING METAMORPHOSIS IN *XENOPUS LAEVIS*

P4	Jeffrey Yoder	THE CONFOUNDING COMPLEXITY OF INNATE IMMUNE RECEPTORS WITHIN AND BETWEEN TELEOST SPECIES
P5	George Heath	COMPARING THE IMMUNE FUNCTION OF TRIPLOID AND DIPLOID CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*): CAN WE MAKE TRIPLOID SALMON IMMUNOCOMPETENT?

19.00-?? Dinner on your own or Informal Lobster dinner

Thursday June 23rd: Lecture Hall A, Atlantic Veterinary College

	Special Session	Comparative Immunology at Work in Atlantic Canada
9.00-9.40	Nellie Gagne	AN INSIGHT ON ATLANTIC SALMON (*SALMO SALAR*) IMMUNE FUNCTIONS AND DISEASE RESPONSE
9.40-10.00	M. Braceland	PROTEOMIC AND BIOCHEMICAL INVESTIGATIONS OF ATLANTIC SALMON (*SALMO SALAR L.)* SERUM DURING PANCREAS DISEASE
10.00-10.20	Tiago Hori	MICROARRAY ANALYSIS OF THE RESPONSE OF ATLANTIC SALMON (*SALMO SALAR*) PRIMARY MACROPHAGES TO INFECTION WITH *PISCIRICKETTSIA SALMONIS*
10.20-10.40	Mark Fast	ACUTE RESPONSIVENESS IN ATLANTIC STURGEON TO PAMPS AND PARASITES
10.40-11.00	Coffee	
		Development of Immunity
11.00-11.20	Bethaney Fehrenkamp	ELUCIDATING MATERNAL INVESTMENT IN PASSIVE IMMUNE TRANSFER THROUGHOUT LACTATION IN A MODEL MARSUPIAL
11.20-11.40	Jacques Robert	DEVELOPMENTAL EXPOSURE TO POLLUTANTS FROM AGRICULTURE AND UNCONVENTIONAL OIL AND GAS EXTRACTION ALTERS METAMORPHOSIS AND INNATE ANTIVIRAL IMMUNITY IN THE AMPHIBIAN XENOPUS
11.40-12.00	Bram Lutton	AMD3100-INDUCED LEUKOCYTE MOBILIZATION IN *LEUCORAJA ERINACEA*
12.00-13.00		Lunch/Business meeting
		Genomics and Transcriptomics
13.00-13.20	Katherine Buckley	PERTURBATION OF IMMUNE STATE IN THE GUT LUMEN INDUCES SYSTEM-WIDE CELLULAR AND TRANSCRIPTIONAL CHANGES IN THE SEA URCHIN LARVA
13.20-13.40	Nicholas Schuch	DIVERSITY IN A PURPLE SEA URCHIN LARVAL MODEL OF MICROBIAL COLONIZATION AND IMMUNITY
13.40-14.00	Fraser Clark	RNA-SEQ: A POWERFUL TOOL FOR IMMUNE PATHWAY DISCOVERY IN NON-MODEL

		ORGANISMS
14.00-14.20	Elisa Casadei	INFLUENCE OF MICROBIOME DURING OLFACTORY EPITHELIUM DEVELOPMENT
14.20-14.40	Sara Purcell	DEVELOPMENT OF A MULTIPLEX NUCLEIC ACID ASSAY FOR SALMON IMMUNOLOGICAL ASSESSMENT
14.40-15.00	Thad Deiss	DEEP SEQUENCING OF *B. TAURUS* IMMUNOGLOBULIN HEAVY CHAIN REPERTOIRE
15.00-15.20	Coffee	
		Innate immunity: Antiviral and PRRS
15.20-15.40	J. Le Nours	ATYPICAL NATURAL KILLER T-CELL RECEPTOR RECOGNITION OF CD1D-LIPID ANTIGEN
15.40-16.00	Andrea Monjo	DSRNA SENSING IN FISH: USING CHSE-214 AS A MODEL FOR STUDYING CLASS A SCAVENGER RECEPTORS
16.00-16.20	Courtney Smith	SPTRANSFORMER, A MULTITASKING RECOMBINANT SP185/333 PROTEIN FROM THE PURPLE SEA URCHIN BINDS TO MULTIPLE TARGETS
16.20-16.40	Graham Lisser	UNDERSTANDING THE INNATE ANTIVIRAL IMMUNE RESPONSE TO FROG VIRUS 3 (FV3)
16.40-17.00	Louise-Marie_Roux	LOBSTERS IN HOT WATER: IMPACT OF TEMPERATURE ON THE AMERICAN LOBSTER'S MOLECULAR IMMUNE RESPONSE TO WSSV
17.00-17.20	Sarah Poynter	*IN VITRO* TRANSCRIBED DSRNA AS A SURROGATE FOR NATIVE DSRNA IN RAINBOW TROUT CELLS
17.20-17.40	John Pham	CHARACTERISATION OF AN ATLANTIC SALMON HEART ENDOTHELIAL (ASHE) CELL LINE AND EVALUATING ITS ABILITY TO SUPPORT FISH VIRUS INFECTION

19.00-22.00 Banquet PEI Brew Company

Abstracts

Weds June 22nd, 9.05: Early evolutionary development of adaptive immunity

REDEFINING GENE DIVERSIFICATION DOGMAS: LESSONS FROM THE IMMUNE SYSTEM OF ECHINODERMS

Matan Oren[1], Benyamin Rosental[2], Megan A. Barela Hudgell[1], Brian D'Allura[1], Jacob Agronin[1], Daniele Podini[3], L. Courtney Smith[1]

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[3] The Department of Forensic Sciences, The George Washington University, Washington DC

Over evolutionary time, immune systems in a wide range of organisms have acquired different gene diversification mechanisms to adapt to changing environments and rapidly evolving pathogens. One of the most successful mechanism is the V(D)J somatic recombination which give rise to the enormous diversity of Immunoglobulins and T cell receptors. So far, this type of adaptive immunity has been found only in higher vertebrates and its sudden evolutionary appearance is still enigmatic. We are studying an immune gene family in the California purple sea urchin; the *Sp185/333*. This gene family exemplifies unique structural features including modular exonal units, gene clustering, microsatellites surrounding genes, segmental duplications and allelic mispairing. We found that single sea urchin cells express a single *Sp185/333* message. We also identified different genomic *Sp185/333* repertoires among different individuals and among single cells within an individual. These finding suggest that, in similar to the vertebrate adaptive immune system, *Sp185/333* gene family is going through genomic rearrangements to modify the expression of its members. Interestingly, the purple sea urchin, express all basic genes required for V(D)J recombination including RAG1 and RAG2. We also identified two clusters of recombination signal sequences (RSS) in proximity to the *Sp185/333* genes. We therefore suspect that a vertebrate-like RAG complex is involved in the *Sp185/333* DNA rearrangements as well as other unknown mechanisms.

Weds June 22nd, 9.35: Early evolutionary development of adaptive immunity

Identification of novel immune mediators in the transcriptome of *Strongylocentrotus purpuratus* coelomocytes

Mei-Chen Liu1, Wen-Yun Liao1, Katherine M. Buckley5, and Jonathan P. Rast5 and Sebastian D. Fugmann1,2,3,4

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The genome of the purple sea urchin (*Strongylocentrotus purpuratus*) contains a number of genes whose products have previously been implicated as important mediators of adaptive immunity. This includes the *S. purpuratus* homologs of the recombination activating genes 1 and 2 (*SpRag1L and SpRag2L*) whose vertebrate cousins are critical for the assembly of the antigen receptors genes that are a hallmark of adaptive immunity. These findings suggest that echinoderms are placed at an important transition point between innate and adaptive immunity. To gain insight into the function of sea urchin immune cells we established fluorescently-labeled lectins as molecular markers for distinct coelomocyte populations and initiated a RNAseq-based analyses of the transcriptomes of the four most abundant coelomocyte types. Here we will also discuss a recent finding from an ab initio transcriptome assembly approach that reveals a novel insight on the evolution of DNA diversification processes in vertebrate immunity.

Weds June 22nd, 10.05: Early evolutionary development of adaptive immunity

SOMATIC HYPERMUTATION AND USE OF IMMUNOGLOBULIN VARIABLE REGIONS AT SHARK T CELL RECEPTORS

Michael F. Criscitiello1, Jeannine O. Eubanks1, Thaddeis C. Deiss1, Patricia L. Chen1, Caitlin D. Castro2, Yuko Ohta2Martin F. Flajnik2

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The nurse shark (*Ginglymostoma cirratum*) is part of the earliest clade found to possess an adaptive immune system employing immunoglobulin superfamily lymphocyte antigen receptors. The hallmarks of adaptive immunity, memory and specificity, are accomplished through B or T cells employing immunoglobulin (Ig) or T-cell receptors (TCR) respectively. Both lineages undergo somatic gene rearrangement for receptor diversification, a process mediated by RAG proteins, in which V, D, and J gene segments are joined together. Surprisingly, we found somatic hypermutation (SHM, normally a B cell process) to occur extensively at shark TCRα loci. Unlike B cell SHM that follows antigen exposure in sharks and most other vertebrates, SHM at TCRα loci occurs during thymic development. TCRα SHM is supported with isolation of clusters of mutated clones having the same CDR3 rearrangement. We suggest that such SHM, in addition to TCRα receptor editing, permits developing T cells with evolving receptors to scan cortical epithelial cells for positive selection on self-peptide/self MHC. Additionally, we have found that shark TCRδ can employ variable gene segments of IgM and IgW in rearrangements, a situation that may be similar to the IgHV use by TCRδ noted in higher vertebrates. Future studies must further study the lineage commitment of shark B and T cells and the receptor plasticity of these lineages in all vertebrates.

Weds June 24th 11.10: Pathogens

Vaccine-induced protection against bacterial infections in Arctic charr (*Salvelinus alpinus*)

Shona Whyte[1*], Alyson Brown[1], Laura Braden[1], Allison MacKinnon [2], Tiago Hori[3], David Groman[3], Mark Fast[1]

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While there is extensive knowledge on salmonid culture in general, in the case of disease prevention, major differences exist across salmonid species in terms of susceptibility to specific pathogens and efficacy of vaccine protection. Arctic charr are susceptible to furunculosis and bacterial kidney disease, serious bacterial diseases that affect the productivity of salmonid farms worldwide. The objective of this laboratory study was to evaluate the efficacy of two commercially available vaccines against furunculosis and BKD in the Fraser strain of Arctic charr. Fish were vaccinated with Forte Micro (Elanco Animal Health), Renogen (Elanco Animal Health) or a combination of both vaccines. Fish were challenged with *Aeromonas salmonicida ssp. salmonicida* by intraperitoneal injection at 400 degree days. Forte Micro and the combination of Forte Micro and Renogen showed significantly greater efficacy against *A.salmonicida* ssp. *salmonicida*. There also appeared to be a slightly better efficacy with the combination of Forte Micro and Renogen than with the Forte Micro alone. Characterization of the transcriptomic response to vaccination and infection was investigated using head kidney samples. Forty-eight individual libraries were prepared using the TruSeq mRNA stranded library preparation kit for high quality total RNA. Samples were barcoded and sequenced in 4 lanes of a HiSeq2000 sequencer (12 samples per lane). Resulting reads were trimmed for adaptors and quality and a de-novo transcriptome assembly was generated using the Trinity v2.01 assembler. Following assembly, reads for each individual were mapped back to the transcriptome using kallisto v0.42.4 to quantify gene expression. Differential gene expression was evaluated using edgeR. Among the many differences detected, 148 genes (96 up-regulated and 52 down-regulated) were differentially expressed between the fish injected with Forte Micro and the Forte Micro+Renogen combination following vaccination and, 131 genes (72 up-regulated and 59 down-regulated) between the same groups at the on-set of mortality.

Weds June 24th 11.30: Pathogens

USING OUTBREEDING TO IMPROVE THE IMMUNOLOGICAL PERFORMANCE OF CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTCHA)* IN RESPONSE TO *VIBRIO ANGUILLARUM*.

S.L. Semple[1], D.D. Heath[2] and B. Dixon[1].
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Although the aquaculture industry in North America is currently dominated by Atlantic salmon, there has been an increasing interest in the production of species native to the Pacific coast such as Chinook salmon (*Oncorhynchus tshawytcha*). Because Chinook salmon is relatively new to culture production, the selection and propagation of appropriate stocks is critical for the success of this developing species. In many cases genes from wild populations are incorporated into farmed stocks to avoid the decrease in performance that is associated with inbreeding. The current study focuses on assessing the immunological performance of an inbred aquaculture stock along with 7 different hybrid stocks of Chinook salmon after exposure with the marine pathogen, *V. anguillarum*. Following disease challenge these hybrid groups showed a large degree of variability in mortality throughout infection indicating that certain crosses were able to combat bacterial infection more effectively than others. To further explore this variation in disease resistance, antibody development, cytokine expression and MHC alleles will also be compared between the hybrid crosses. Understanding the impact of outbreeding on the immune function of farmed, and often inbred, Chinook salmon could aid in the development of high-quality aquaculture stocks for this species.

Weds June 24th 11.50: Pathogens

AN IMMUNOGENIC PEPTIDE DERIVED FROM THE RIBOSOMAL PROTEIN P0 AS VACCINE CANDIDATE FOR THE CONTROL OF SEA LICE INFESTATIONS

Yamila Carpio[1], Janet Velazquez[1], Yeny Leal[1], Naylin Herrera[1], Claudia García[1], Jannel Acosta[1], Antonio Morales[1], Fumio Takizawa[2], Oriol Sunyer[2], Mark Fast[3], Mario Pablo Estrada[1**]
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[2]School of Veterinary Medicine, University of Pennsylvania, USA
[3]Atlantic Veterinary College, University of Prince Edward Island, Canada

Sea lice (*Copepoda, Caligidae*) are the most widely distributed marine pathogens in the salmon industry. In this context, vaccination could be an efficient, environmentally safe and economically sustainable alternative for parasite control. Although emerging sea lice proteins have been identified recently as potential targets for generating protective molecules, only a limited number of them have been evaluated in vaccine trials with unsuccessful results. The protein P0 is essential for the assembly of the 60S ribosomal subunit and essential for cell viability. A vaccination-challenge trial with an immunogenic peptide of *Rhipicephalus sanguineus* protein P0 reduced survival of ticks with an overall efficacy of 90%, suggesting that it might be a promising antigen candidate for the control of ectoparasite. We have identified an immunogenic region of the ribosomal protein P0 from *Caligus rogercresseyi* and *Lepeophtheirus salmonis* that is not very conserved compared to host P0. We developed several vaccine candidates based on this peptide and produced them in *E. coli*. These antigens were able to elicit a high specific IgM antibody response after intraperitoneal (ip) immunization measured by ELISA using tilapia as teleost fish model. Additionally, we isolated and cloned for first time IgT, IgM, IFN-γ and IL-4 cDNA fragments with the aim to develop antibodies to monitor tilapia immune response to vaccination and evaluate the impact of our vaccinate candidates on the fish immune system. These findings will be finally validated in an immunization-challenge trial in *Salmo salar*. The overall results are relevant in the development of an effective vaccine against sea lice.

KEYWORDS
Sea lice, vaccine, immune response, cytokines, ribosomal protein

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Weds June 24th 12.10: Pathogens

IMMUNOTOXICITY OF GEOGRAPHICALLY DIVERSE ISOLATES OF A PATHOGENIC AMPHIBIAN CHYTRID FUNGUS

Louise Rollins-Smith[1,2,3], Heather L. Wells[1], Shawna K. McLetchie[1], Savannah L. Alford[2], F. Ann Sobell[1], and Laura K. Reinert[1].
[1]Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN; [2]Department of Biological Sciences, Vanderbilt University, Nashville, TN; [3]Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN.

Pathogenic fungi that infect vertebrates have complex interactions with their hosts, and immune defenses usually keep them in check. However, many fungi have evolved immune evasion strategies. Previous work has shown that the pathogenic amphibian chytrid, *Batrachochytrium dendrobatidis (Bd)*, releases factors that inhibit proliferation and induce apoptosis of normal amphibian B- and T-lymphocytes and normal and neoplastic mammalian lymphocytes. Other recent studies have revealed considerable genetic variability in isolates of *Bd* from different locations around the world. Here we used a standard proliferation assay and a colorimetric MTT assay to compare the capacity of *Bd* isolates from different geographic locations to inhibit proliferation of the human T cell line, Jurkat. Most isolates tested inhibited Jurkat cell viability by about 50 % (range 39 to 61% at a 5-fold supernatant concentration), but a few isolates were significantly more inhibitory or less inhibitory. This suggests that isolates vary in their capacity to inhibit immunity which may be linked to virulence in their amphibian hosts.

Weds June 24th 13.00: Regulation and Macrophage

Gene regulatory network control of immune system development and response

Jonathan P. Rast [1,2,3], Katherine M. Buckley [1,2,3], Catherine Schrankel [2,3], Nicholas Schuh [1,3], and Eric Ho [1,3]

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Gene regulatory network approaches have been especially useful in characterizing control of embryonic development but they can also be applied to immunity. The sea urchin larva has a relatively simple morphology, yet displays a complex response to gut-associated microbial disturbance that involves changes in the activity of hundreds of genes expressed throughout the organism as well as complex interactions among several types of differentiated immune cells and the cells of the gut epithelium. We have developed a model to characterize this response using the marine bacterium *Vibrio diazotrophicus*. Immune factors are expressed in many tissues and involve homologs of (1) factors that are also important in vertebrate immunity (*e.g.*, PU.1, IL-17), (2) response genes that are specific to sea urchins (*e.g.,* 185/333) and (3) an interesting set of novel genes with homologs that are widely distributed in bilaterians, but absent in vertebrates and ecdysozoan model organisms. The morphological simplicity of this system provides a model to investigate system-wide molecular interactions at single-cell resolution and to characterize the gene regulatory network that underpins organismal immunity. We are now addressing the mechanisms that control the development of the distributed immune system in embryogenesis and how immune activity feeds back into the development of larval immune cells. By focusing on the interactions among regulatory DNA, the transcription factors that carry information from one gene to another, and the signal systems that convey cell state from nucleus to nucleus, we can begin to unravel complex organism-wide control of immunity in this simple deuterostome model.

Weds June 24th 13.20: Regulation and Macrophage

FIRST IDENTIFICATION OF REGULATORY B CELL SUBSETS EXPRESSING IL-10 IN A NON-TETRAPOD SPECIES

Fumio Takizawa[1], Tomas Korytar[1], Yasuhiro Shibasaki[1], Daniela Gómez[1], Zhen Xu[1], David Parra[1], Gregory D. Wiens[2], J. Oriol Sunyer[1]

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IL-10 is an immunoregulatory cytokine with a potent anti-inflammatory activity. IL-10 is produced by variety of cells, including antigen presentation cells and T cells. Notably, mammalian B cells producing IL-10, referred to as regulatory B (Breg) cells, have recently attracted considerable attention due to their immunosuppressive roles in autoimmunity, inflammation and tumorigenesis. In teleost, IL-10 has been identified in some fish species including *Cyprinidae* and *Salmonidae*. However, the cell types producing transcripts and proteins of IL-10 are largely unknown in teleosts. To determine whether teleost have B cell subset homologous to mammalian Breg cells, we sorted IgM^+ and IgT^+ B cells from rainbow trout infected with *Yersinia ruckeri* or *Ceratomyxa shasta* and analyzed the transcription levels of IL-10 in these B cell subpopulations. Moreover, we developed rabbit antibodies against recombinantly produced rainbow trout IL-10 to detect the presence of IL-10-producing B cells in lymphoid tissues. In *Y. ruckeri* infected fish, spleen IgT^+ but not IgM^+ B cells induced statistically significant higher IL-10 transcripts when compared to the same B cell subsets of control fish. Moreover, IgT^+ B cells represented the spleen leukocyte population expressing the highest IL-10 transcript levels. More critically the presence of IgT^+ B cells expressing IL-10 could be confirmed by immunohistochemistry as well as by flow cytometry. These results imply for the first time the existence of IL-10 producing B cells in a non-mammalian species, and establish the basis for studying the role and evolution of regulatory B cells using teleosts as model species.

Weds June 24th 13.40: Regulation and Macrophage

A CONSERVED ALTERNATIVE FORM OF THE PURPLE SEA URCHIN HEB/E2-2/E2A TRANSCRIPTION FACTOR MEDIATES A SWITCH IN E-PROTEIN REGULATORY STATE IN DIFFERENTIATING IMMUNE CELLS

Catherine S. Schrankel[1], Katherine M. Buckley[1], Cynthia M. Solek[2], Michele K. Anderson[1] and Jonathan P. Rast[1]

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Integral to many aspects of hematopoietic stem cell biology and development are E-proteins, a family of basic helix-loop-helix (bHLH) transcription factors. E-proteins dimerize with other bHLH factors (*e.g., SCL/TAL2/LYL1* and *Id*) and function in multi-protein complexes with *Gata* factors. Among the vertebrate E-proteins (*E2A/TCF3*, *HEB/REB/TCF12* and *E2-2/ITF-2/TCF4*), the HEB and E2-2 loci contain unique, internal alternative (Alt) starts of transcription. The truncated Alt isoforms are under separate regulatory control than ubiquitous canonical (Can) forms. However, the specific contributions of Alt E-proteins are poorly understood, given the combinatorial diversity of binding partners and cellular complexity in vertebrates. We use the purple sea urchin embryo to explore the gene regulatory networks that control immune cell specification and differentiation. Notably, sea urchins have single orthologs of all relevant factors involved in mammalian E-protein biology (*SpGata, SpScl,* and *SpId*). These factors specify immune-cell precursors in a network similar to vertebrate stem cells. Furthermore, the *SpE-protein* locus is organized identically to vertebrate *HEB* and *E2-2* and contains a homologous E-Alt form (*SpE-Alt*). In contrast to the widely expressed canonical form (*SpE-Can*), *SpE-Alt* expression is tightly restricted to immune-cell precursors in the embryo. Perturbation studies indicate that *SpE*-Alt is required for the development and migration of immunocytes. Fluorescent reporters and in-situ analyses demonstrate that *SpE-Can* becomes excluded from *SpE-Alt*$^{+}$ differentiating cells. This is dependent on SpE-Alt function, and represents a novel negative feedback between the two E-protein forms. Our work highlights both novel and fundamental properties of E-protein biology that can be identified with comparative approaches.

Weds June 24th 14.00: Regulation and Macrophage

CHARACTERIZATION OF EVOLUTIONARILY CONSERVED CD4$^+$ MONOCYTES/MACROPHAGES IN RAINBOW TROUT

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In addition to helper T cells, mammalian CD4 is expressed on a variety of cell types, including macrophages. Teleost fish possess two CD4 molecules, CD4-1 and CD4-2, however their expression on myeloid cells remain to be characterized. The goal of this study was to identify the CD4$^+$ subset within the myeloid leukocyte population in rainbow trout. Staining of head kidney cells with anti-CD4-1 and anti-CD4-2 mAbs identified a significant population of myeloid cells with only CD4-1 surface expression. Gene expression analysis revealed that CD4-1$^+$ myeloid cells expressed high transcript levels of monocyte/macrophage markers. In contrast, the same cells expressed negligible amounts of *mpo*, a neutrophil marker. Conversely, CD4-1$^-$ myeloid cells expressed high levels of *mpo* while expressing very low to negligible transcript levels of the monocyte/macrophage markers. We then used cytochemical staining to characterize further the identity of CD4-1$^+$ myeloid cells. Most of these cells were positive for β-glucuronidase and naphthol AS-D chloroacetate esterase staining while negative for myeloperoxidase (MPO) and Sudan Black B (SBB) stains. In contrast, CD4-1$^-$ myeloid cells included mostly polymorphonuclear cells that stained positively with MPO and SBB. These analyses strongly suggest that CD4-1$^+$ myeloid cells are monocytes/macrophages while CD4-1$^-$ myeloid cells mostly comprise neutrophils. Furthermore, we found that CD4-1$^+$ monocytes/macrophages represent the myeloid population with the highest phagocytic activity and capacity. Our data represent the first description of CD4$^+$ monocytes/macrophages in a non-mammalian species, thus suggesting that CD4 expression in these cells is the result of an ancient evolutionary event preceding the emergence of tetrapods.

Weds June 24th 14.20: Regulation and Macrophage

Evaluating phagocytic ability of RTS11-GFP, a stably transfected sub-line of RTS11, a monocyte/macrophage cell line from spleen of Rainbow Trout

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Cell lines derived from monocytes/macrophages have been notoriously hard to transfect and few fish cell lines have been stably transfected with foreign genes. RTS11 is a monocyte/macrophage cell line derived from spleen of rainbow trout that has been in use for almost 20 years. This cell line has been widely used and has been instrumental in understanding many aspects of fish innate immunity. In November of 2009, RTS11 were transfected with pmaxGFP® using Amaxa's Nucleofection® process. Various trade-marked Nucleofector® programs were used and transfection was achieved in over 80% of the treated cultures with cell viability ranging from 50 to 100% and transfection success close to 40% of cells. Treatment groups with the highest transfection efficiencies were clonally subcultured into 96 well multiplate dishes and 4 clones were selected that had greater than 90% expression of GFP. These were passaged into 12.5 cm flasks and upon confluency in suspension, were passaged into larger flasks resulting in the current RTS-11GFP subline which has now been passaged for over 6 years. This subline has been frozen and thawed successfully and have been used in phagocytosis experiments testing for effects of neonicotinoid insecticides on ability of cells to engulf fluorescence particles. Neonicotinoids appear to stimulate phagocytosis in a dose dependent manner but expression of GFP protein declined with increased phagocytosed particles. RTS11-GFP could be excellent model systems for studies of phagocytosis and as indicators for toxicant exposure.

Weds June 24th 14.40: Regulation and Macrophage

DISRUPTION OF TRIM9 FUNCTION ABROGATES MACROPHAGE MOTILITY *IN VIVO*

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The vertebrate immune response is comprised of multiple molecular and cellular components that interface to provide an adequate defense against pathogens. Although much is known on how individual molecules or cells respond to infection, an understanding of the whole-organism response to pathogen exposure remains unresolved, due to the dynamic complexity of the immune system and its interdependent innate and adaptive functionality. Zebrafish larvae provide a unique model for overcoming this obstacle as larvae can successfully defends themselves from pathogens while lacking a functional adaptive immune system during the first few weeks of life, making it possible to examine exclusively the innate immune response in a whole-organism context. It was hypothesized that the transcriptional response of zebrafish larvae to immune agonists would identify known immune-response genes as well as reveal genes that mediate innate immunity in novel ways. In order to test this hypothesis, zebrafish larvae were exposure to polyIC and Pam3CSK4 and transcriptome analyses completed using microarray analyses. This strategy successfully identified known immune response genes, as well as genes that had not been implicated in immune function, including the E3 ubiquitin ligase, tripartite motif 9 (*trim9*). Although *Trim9* expression has been described as "brain specific", here we demonstrate elevated levels of *trim9* transcripts in macrophages after immune stimulation. As Trim9 has been implicated in axonal migration, we investigated and demonstrate that disruption of Trim9 function impairs macrophage chemotaxis and cellular architecture in vivo. These results demonstrate that Trim9 mediates cellular movement and migration in macrophages as well as neurons.

Weds June 24th 15.20: Self Recognition: MHC, TCR and more

Autoimmunity and immune recognition in the *Drosophila melanogaster tuSz* mutant

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Drosophila melanogaster larvae are commonly infected by parasitoid wasps and mount a robust cellular immune response following parasitization. This immune response culminates in the melanotic encapsulation of the wasp egg, however, the molecular mechanisms by which fly immune cells recognize the wasp egg as foreign are unknown. Flies mount a similar encapsulation response against xenografts or abiotic objects, suggesting that rather than non-self recognition, the melanotic encapsulation response may be initiated by 'missing-self' recognition. To gain insight into the mechanisms underlying immune recognition, we are using molecular genetic approaches to characterize an autoimmune *D. melanogaster* mutant, *tumor(1)Suzuki* (*tuSz*), in which flies mount a self-directed cellular immune response, presumably due to the absence of, or the inability to recognize, the 'self' signal. We found that the *tuSz* autoimmune phenotype genetically maps to two closely linked loci, one that leads to ectopic activation of the cellular immune response, and a second that disrupts protein N-glycosylation. Interestingly, either of these changes fail to produce an autoimmune phenotype in isolation, suggesting a synergism between immune cell activation and altered or missing self. These findings demonstrate that *D. melanogaster* immune recognition is dependent on protein N-glycosylation, and that this mark is recognized by immune cells following activation of the cellular immune response.

Weds June 24th 15.40: Self Recognition: MHC, TCR and more

RESOLUTION OF *KUDOA THYRSITES* INFECTION IS ASSOCIATED WITH INFILTRATION OF MHIIβ+ CELLS IN ATLANTIC SALMON, *SALMO SALAR*

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Kudoa thyrsites is a myxozoan parasite of the skeletal muscle in a wide range of fish hosts with a global distribution. In British Columbia, Canada, infections in farmed Atlantic salmon (*Salmo salar*) incur significant economic losses due to post-mortem myoliquefaction. Despite obvious commercial importance, little is known about the life-cycle or host-parasite relationship of *K. thyrsites*. Atlantic salmon can recover from experimental infections and the recovery process is characterized by a gradual loss of plasmodium structure and replacement with fibrous connective tissue. The cellular mechanisms responsible for this process are not known although macrophage-like phagocytes containing mature spores have been observed in chronically infected fish. To address the possibility of protective adaptive immunity in recovered fish, Atlantic salmon were exposed to infective seawater for 500 or 1000 degree-days (DD). The fish were maintained in UV-sterilized seawater and muscle samples were examined histologically at 2000, 3500 and 4312 DD. Previously exposed fish and unexposed controls were exposed to seawater-containing *K. thyrsites* spores, from 4312 to 4812 DD and histological examinations conducted at 6312 DD. Prevalence and severity of *K. thyrsites* declined significantly between 2000 and 4312 DD and there was no statistical difference between the exposure groups. Following re-exposure, the prevalence and severity of infection were significantly lower in previously exposed salmon compared with controls. Significant infiltration of MHIIβ+ cells was detected in the musculature of infected salmon compared to uninfected salmon. The association of these cells with infected myocytes proceeded in 4 stages: initial contact and envelopment of the myocyte, infiltration of the myocyte, envelopment of the plasmodium and complete degradation of the plasmodium and dissemination of spores by positive cells. Transcriptional profiling of infect muscle tissue showed concordant up-regulation of *MHII*, accompanied by significant up-regulation of *IgMm* and *IgT*. Other cellular markers (*CD4, CD8, CD83*) are currently being profiled to help characterize the cellular response during detection and resolution of Atlantic salmon with *K. thyrsites*.

Weds June 24th 16.00: Self Recognition: MHC, TCR and more

REVERSE GENETIC ANALYSIS OF *XENOPUS LAEVIS* NONCLASSICAL MHC CLASS IB GENES BY CRISPR/CAS9-BASED GENOME EDITING.

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A large family of 23 *Xenopus* nonclassical MHC class Ib (*XNC*) genes determines *Xenopus laevis* immunity and potentially its physiology. Using RNA interference technology, we previously demonstrated that one of *XNC* genes, *XNC10.1*, is critical for the development and function of specialized innate T (iT) cells, expressing an invariant T cell receptor (TCR) rearrangement – Vα6-Jα1.43. For accelerated, more reliable, and consistent functional studies of *XNC* genes, we implemented a CRISPR/Cas9-based gene disruption approach. Despite an unanticipated high level of somatic mosaicism, we efficiently and specifically generated single gene knockouts of *XNC10.1*, *XNC11*, and *XNC1* as well as double gene knockouts of *XNC10.1* and *XNC11* in *X. laevis*. The absence of transcripts for *XNC10.1* and *Vα6-Jα1.43* TCR in *XNC10.1* knockouts *X. laevis* tadpoles indicated XNC10.1 loss-of-function and deficiency in innate Vα6-Jα1.43 iT cells. Surprisingly, *XNC1* gene disruption induced mortality during a larval developmental stage 47, suggesting some non-immune but essential function of this gene. These data demonstrated that the CRISPR/Cas9 system can be successfully adapted for a rapid genetic analysis of *XNC* gene family.

Weds June 24th 16.20: Self Recognition: MHC, TCR and more

Developing a model in Xenopus to study nonclassical MHC Ib-restricted innate T cells in macrophage-mediated anti-Mycobacterium marinum responses

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Increasing evidence suggests that non-classical MHC (class Ib)-restricted innate (i)T cells play important roles during early stages of bacterial infections. Unlike conventional T cells that express a broad T cell receptor (TCR) repertoire, these iT cells have invariant TCRα rearrangements. In the amphibian *Xenopus* tadpoles, 6 different predominant invariant TCRα rearrangements have been identified, indicative of six distinct iT populations. Using RNAi loss-of-function by transgenesis, we have shown that *XNC4* is required for the development of a specific iT cell population expressing the invariant TCR Vα45-Jα1.14 rearrangement. Interestingly, *Xenopus* tadpoles have natural deficiency of classical MHC class Ia (class Ia) function unlike adults that are both XNC and class Ia competent. Since tadpoles are still capable of responding against pathogens, it is thought that various *Xenopus* non-classical MHC (*XNC*) molecules control the development and function of a dominant iT cell-mediated immunity in tadpoles. To investigate the role of *XNCs* in anti-bacterial immunity, we have developed an infection model with *Mycobacterium marinum (Mm)*, a natural pathogen for *Xenopus*, fish, and humans. We found that tadpoles are as resistant as adult frogs to *Mm* infection, whereas *XNC4*-deficient tadpoles are markedly more susceptible. Collectively, these results suggest that XNC4-restricted iT cells are critical for anti-*Mm* immunity in *Xenopus*. We anticipate that further study with this model will provide new fundamental insights into the roles of class Ib-restricted iT cells during mycobacterial infections.

Weds June 24th 16.40: Self Recognition: MHC, TCR and more

CHARACTERIZATION OF T CELL RECEPTOR MOLECULES IN AFRICAN LUNGFISH (*Protopterus dolloi*)

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T cells are a main arm of the adaptive immune system of jawed vertebrates. T cells perform a variety of roles to defend against invading pathogens and have the ability to differentiate into unique effector functions tailored to best eliminate the target pathogen. T cells are characterized by bearing a T cell receptor (TCR) on their surface. There are four main TCR chains that form two main variations to the TCR: αβ TCR and γδ TCR. In mammals, T cells with a αβ TCR are mostly systemic T cells that circulate throughout the body, while γδ T cells are localized to the epithelial surfaces (i.e. gut and skin), recognize unique antigens with little variability and are involved in wound healing. Studies have shown that cartilaginous fish have a higher distribution of γδ T cells in the systemic compartment, thus differing from mammals. The goal of the present study is to characterize the four TCR chain in the African lungfish (*Protopterus dolloi*), the closest extant relative to all tetrapods. Phylogenetic analyses show that lungfish TCR molecules resemble more those of tetrapods than teleost or cartilaginous fish TCRs. Upon in vivo bacterial infection, lungfish δ chain expression was up regulated in a variety of systemic and mucosal lymphoid tissues suggesting that the dichotomy of TCR αβ and γδ may have emerged in endotherms and is not present in sarcopterygian fish. Future studies will further establish the role of γδ T cells in lungfish skin in response to wounding or UV damage.

Weds June 24th 17.00: Posters

P1:

Two different prominent nonclassical MHC class I-restricted invariant T cell lineages with non-overlapping critical antiviral and anti-mycobacterial immune functions in the amphibian Xenopus

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Unconventional T cells, including disparate populations of MHC class Ib-reactive innate T (iT) cells are emerging as key factors in the immune system partly due to their public antigen specificities and rapid effector responses. The biological relevance and evolutionary conservation of iT cells have, over the past few years, been strengthened by studies in the amphibian *Xenopus,* which have revealed an overrepresentation of several invariant TCRs in tadpoles and identified a prominent subset of iT cells (invariant Vα6 [iVα6]) restricted by the MHC-like molecule XNC10. Recently, we showed that similar to CD1d restricted iNKT cells in humans and mice, *Xenopus* iVα6 T cells are critical for early antiviral immunity. Here, using RNAi loss-of-function by transgenesis targeting another *Xenopus* nonclassical gene, XNC4, we have identified a different XNC4-dependent iT cell population expressing one of the 6 previously identified overrepresented TCRα rearrangements (TRAV45 joined to TRAJ1.14). We show that this invariant Vα45 [iVα45] T cell population is critical for antibacterial immunity against *Mycobacterium marinum.* These data suggest that functionally distinct populations of MHC class Ib-reactive iT cell populations play a prominent role in amphibian immune defense and as such may represent a more primordial immune cell type that previously thought.

P2:

Following the Humoral Immune Response in Native New England Amphibians Using Reagents Developed in *Xenopus laevis.*

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Amphibian decline is a global phenomenon that defies any single explanation but is frequently attributed to disease. Amphibian mortality may be evidence of environmental factors that adversely affect immune function. Much study has been devoted to the immune response of the South African clawed frog *Xenopus laevis*, however much less research has been focused on other amphibian species, many of which have experienced precipitous population declines. One such amphibian, *Lithobates pipiens* (the northern leopard frog) was once found in large numbers in northern North America. It appears now that their numbers are dwindling as are those of many other amphibian species.

One facet of the complex vertebrate immune system is the humoral response. The initial objective of these experiments was to verify that reagents developed in *Xenopus* can be utilized to follow the humoral response of native anuran amphibians. We utilized an ELISA to detect antibodies produced by several species of New England amphibians. Amphibian humoral immune responses begin with the production of IgM and re-exposure to an immunogen results in an isotype switch to IgY. We used our ELISA to successfully follow the appearance of IgM and IgY in the serum of native amphibians using reagents developed in *Xenopus laevis.*

Our experiments yielded an additional finding: in frogs, the secondary immune response included the production of IgY as expected as well as a substantial IgM component. We describe this as an incomplete isotype switch and demonstrate that it is characteristic of the humoral response of both *L. pipiens* and *Xenopus*.

P3:

DEVELOPMENTAL EXPRESSION PROFILES AND THYROIDAL REGULATION OF CYTOKINES DURING METAMORPHOSIS IN *XENOPUS LAEVIS*

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Key words: cytokine, thyroxine, sodium perchlorate, innate immune system

Early life-stages of amphibians rely on the innate immune system for defense against pathogens. Thyroid hormones (TH) are critical for metamorphosis and later development of the adaptive immune system; however, the role of TH in innate immune system development is less clear. An integral part of the innate immune response are pro-inflammatory cytokines – effector molecules that allow communication between components of the immune system. The objective of this study was to characterize the expression of key pro-inflammatory cytokines, tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β) and interferon-γ (IFN-γ) throughout amphibian development and determine the impacts of thyroidal modulation on their expression. *Xenopus laevis* were sampled at various stages of development encompassing early embryogenesis to completed metamorphosis and cytokine expression was measured by real-time PCR. Expression of TNFα and IL-1β were transient over development, while IFN-γ remained relatively stable. Athyroid, pre-metamorphic tadpoles were exposed to thyroxine (0.5 and 2μg/L) or sodium perchlorate (125 and 500μg/L) for 7 days. Developmental staging revealed that thyroxine exposure advanced development whereas sodium perchlorate delayed tadpole development (although to a lesser degree) and increased thyroid gland area and follicular cell height. Tadpoles exposed to thyroxine tended to have higher cytokine expression whereas tadpoles exposed sodium perchlorate tended to have lower expression – however, we could not discount the effect of treatment on stage when interpreting results. It appears that thyroidal modulation of cytokines expression is due to indirect effects of treatment on the stage of development rather than a direct effect on these signaling molecules.

P4:

THE CONFOUNDING COMPLEXITY OF INNATE IMMUNE RECEPTORS WITHIN AND BETWEEN TELEOST SPECIES

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Teleost genomes encode multiple multigene families of immunoglobulin domain-containing innate immune receptors (IIIRs) with unknown function and no clear mammalian orthologs. However, the genomic organization of IIIR gene clusters and the structural and signaling motifs of the proteins they encode are similar to those of mammalian innate immune receptor families such as the killer cell immunoglobulin-like receptors (KIRs), leukocyte immunoglobulin-like receptors (LILRs), Fc receptors, triggering receptors expressed on myeloid cells (TREMs) and CD300s. Teleost IIIRs include novel immune-type receptors (NITRs); diverse immunoglobulin domain containing proteins (DICPs); polymeric immunoglobulin receptor-like proteins (PIGRLs); novel immunoglobulin-like transcripts (NILTs) and leukocyte immune-type receptors (LITRs). The accumulation of genomic sequence data has revealed that IIIR gene clusters in zebrafish display haplotypic and gene content variation. This intraspecific genetic variation, as well as significant interspecific variation, frequently confounds the identification of definitive orthologous IIIR sequences between teleost species. Nevertheless, by defining which teleost lineages encode (and do not encode) different IIIR families, predictions can be made about the presence (or absence) of specific IIIR families in each teleost lineage. It is anticipated that further investigations into available genomic resources and the sequencing of a variety of multiple teleost genomes will identify additional IIIR families and permit the modeling of the evolutionary origins of IIIRs.

P5:

COMPARING THE IMMUNE FUNCTION OF TRIPLOID AND DIPLOID CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*): CAN WE MAKE TRIPLOID SALMON IMMUNOCOMPETENT?

S.L. Semple[1], G. Heath[1], D.D. Heath[2] and B. Dixon[1].
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Capture fishery production peaked in 1993 and thus the increasing demand for human consumption of fish can only be met through aquaculture. Atlantic salmon dominates North American aquaculture, but on the Pacific coast of Canada there has been an increasing interest in culturing native species such as Chinook salmon. A major problem in aquaculture is maturation, the onset of which can reduce flesh quality before fish reach market size, this causes in financial losses. One way to combat this problem is to triploidize the fish, which prevents maturation altogether. Sterile triploid escapees also pose no ecological risk to wild stocks. The induction of triploidy is a simple process that does not appear to influence fish development or size, however triploid fish suffer increased mortality and disease susceptibility. The increase in gene number and/or dosage associated with triploidy may lead to greater clonal deletion of T cells during development, which may present as decreased immune function. In an effort to quantify T cell numbers, our lab is developing polyclonal antibodies to Chinook salmon CD3. To further explore the effect of gene dosage and allelic diversity on disease susceptibility, MH class II alleles from triploid and diploid families were compared after disease challenge with *Vibrio anguillarum*. As the induction of triploidy has the potential to increase productivity of salmonid aquaculture worldwide, understanding the observed disease susceptibility could lead to more robust stocks of salmon.

Thurs June 23rd, 9.00: Comparative Immunology at work in Atlantic Canada

An insight on Atlantic salmon (*Salmo salar*) immune functions and disease response

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Infectious diseases among fish present an important economic burden for aquaculture and fisheries industries around the world. For example, the Infectious Salmon Anemia Virus (ISAV) is known to infect farmed Atlantic salmon (*Salmo salar*), and results in millions of dollars of lost revenue to salmon farmers. Although improved management and husbandry practices over the last few years have minimized losses and the number of outbreaks, the emergence of new virulent isolates is still a problem threatening the viability and sustainability of these industries. Understanding the host-pathogen interactions at the genetic level over the course of an infection thus remains of strategic importance, for the development of molecular tools and outcome prediction, vaccine development and efficacy trials. Through *in vivo* challenges in a controlled environment, Atlantic salmons have started to reveal how they respond to disease, how they acquire immunity, and more. Using a 32 k cDNA microarray platform (GRASP), and RNA-seq (transcriptomics), we have studied various signaling pathways and immune regulated genes activated or repressed in the Atlantic salmon during the course of an ISAV infection. Gene expression was measured at different time-points, or at different levels of viral load throughout the infection process. As time progressed, many genes involved in key defense pathways were up regulated including MHC type I, Beta - 2 microglobulin and Barrier to autointegration factor. During the latest stage of the infection process, many genes related to oxygen transportation were under-expressed, correlating well with the biological observations of the anemia process occurring prior to death in Atlantic salmon infected with virulent strains of the ISAV. We suggest that various factors including the viral strain, the genetic makeup of the fish, or previous exposure to ISAV, each affect the outcome of the infection process. This presentation will provide a compilation of various projects mainly on ISAV, and our current understanding of Atlantic salmon response to viruses.

Thurs June 23rd, 9.40: Comparative Immunology at work in Atlantic Canada

PROTEOMIC AND BIOCHEMICAL INVESTIGATIONS OF ATLANTIC SALMON (*SALMO SALAR L.*) SERUM DURING PANCREAS DISEASE

Braceland, M.
The Center for Aquaculture Technologies Canada, Souris, PE,

Salmonid alphavirus subtype 3 (SAV3) is one of six SAV subtypes which cause pancreas disease (PD) in Atlantic salmon (*Salmo salar L.*). While resulting in variable mortality rates, infection with SAV3, which is endemic to Norway, has a high economic impact, costing the industry as much as 14.4 million NOK per 500,000 fish per site. This is largely due to chronic morbidity and reduction in fillet quality PD causes. Despite this, while the pathogenesis of PD is well established, little is understood of the change in humoral proteome both in response to SAV infection the clinical manifestations of disease. This study details the change in Atlantic salmon sera during PD through use of a cohabitation disease challenge model with sampling of blood and tissues being performed 0, 2, 3, 4, 5, 6, 8, 10 and 12 weeks post challenge (wpc). Two-dimension electrophoresis separation of sera and subsequent gel image analysis found 72 protein spots that altered significantly during disease progression. Spots were identified via peptide mass fingerprinting with GLM analysis of each spots profile against histopathological scores of tissues being used to characterise both specific (e.g. enolase 3) and general (creatine kinase) markers of pathology and numerous immune response markers (e.g. compliment components). . In addition, through the use of complimentary proteomic and biochemical methodologies details the need for validation of “omic” results and explores the diagnostic/ prognostic potential of identified proteins.

Thurs June 23rd, 10.00: Comparative Immunology at work in Atlantic Canada

Microarray analysis of the response of Atlantic salmon (*Salmo salar*) primary macrophages to infection with *Piscirickettsia salmonis*

Tiago S. Hori[1], Eva Jakob[2], Fred Kibenge[3], Molly Kibenge[3], Simon Jones[4], Jennifer Hall[5], Matthew Rise[5] [1]The Center for Aquaculture Technologies Canada, Souris, PE, [2]Ewos Innovation, Puerto Varas, Chile, [3]University of Prince Edward Island, Charlottetown, PE [4]Department of Fisheries and Oceans, Nanaimo, BC, [5]Memorial University of Newfoundland, St. John's, NL.

Piscirickettsia salmonis (PSAL) is the etiological agent of salmonid rickettsial septicemia (SRS). SRS can cause high levels of mortality and it is responsible for great economic losses in the aquaculture industry. The goal of this work was to investigate the transcriptomic response of primary culture of Atlantic salmon macrophage/monocytes to infection with PSAL. Primary macrophages were separated from head-kidney homogenates of 8 individual fish and cultured in 6-well (10^6 cells per well) plates. In every 6-well plates, 4 wells were infected with 100 µL of PSAL culture and 2 wells received 100 µL of MEM from a clean CHSE-214 culture. Cells were sampled at 2, 6, 24 and 72 hours post-infection (hpi). Transcriptomic data was obtained for the 2 and 24 hpi time point using the a 44K Atlantic salmon microarray. Differentially expressed genes were identified in R. Interleukin-1 beta, NF-kappa B inhibitor alpha, CD83 antigen precursor Cholesterol-25-hydrolase and tumor necrosis factor were identified as differentially expressed at 2 hpi. These transcripts were validated as differentially expressed due to infection with PSAL using QPCR. Lastly, electron microscopy showed PSAL cells within macrophage as early as 2 hpi and in all other time-points except 72 hours. These finds are consistent with the early up-regulation of cholesterol-25-hydrolase, which is in known to inhibit membrane fusion between viruses and host cells. Taken together, these results shed light in the early interaction between macrophages and PSAL and highlight the role of pro-inflammatory cytokines in the response to infection.

Thurs June 23rd, 10.20: Comparative Immunology at work in Atlantic Canada

Acute Responsiveness In Atlantic Sturgeon To PAMPs And Parasites

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Atlantic sturgeon (*Acipenser oxyrhinchus*), among several other sturgeons, are species of economic and ecological importance in Canada and abroad. While still small in scale, aquaculture for the production of caviar and related products has developed for this and other sturgeon species across North America. In order to develop a better understanding of their immunological competence, overall health and resistance to disease, we conducted short-term exposures of juvenile Atlantic sturgeon to common pathogen associated molecular patterns (PAMPS) like LPS, and more complex parasitic infection. Transcriptomic profiling was used to study their responses over 24-96 hours. Acute phase responses, inflammatory and antigen presentation were all commonly impacted. Individual targets tested for validation showed strong shared concordant expression between the qPCR and RNA-SEq. Specifically serotransferrin 1M and serum amyloid A-1 showed strong induction due to LPS at 24 hrs which was maintained through 72 hrs compared to PBS controls. Furthermore, Serotransferrin was significantly upregulated in the gills of Atlantic sturgeon also at 72 hrs post infection with the copepod parasite *Dichelesthium oblongum*. These acute phase markers, which are often used as diagnostic for infection in higher vertebrates, suggest similar timing and responsiveness to acute pathogen associated molecular patterns and possibly active infection in this ancient fish species.

Thurs June 23rd, 11.00: Development of Immunity

ELUCIDATING MATERNAL INVESTMENT IN PASSIVE IMMUNE TRANSFER THROUGHOUT LACTATION IN A MODEL MARSUPIAL

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All mammalian neonates are highly dependent upon milk for nourishment and immune protection. This is especially true for marsupials, a lineage of mammals with a short gestation, limited placental development, and an increased reliance on an extended lactation period. Most newborn marsupials do not receive passive maternal immunity *in utero* and therefore are entirely dependent upon factors within the milk for immune protection until capable of mounting their own response. Early exposure to potential pathogens, prior to the development of a functional immune system, requires a complex strategy for providing immunological protection. Preliminary sequencing results revealed two distinctive peaks in expression of immune transfer related genes within the mammaries throughout the course of lactation. Based on preliminary data, additional time points have been investigated using quantitative real-time PCR methods, and a more comprehensive transcriptome has been created from time points identified as critical in maternal investment in the developing immune system. Preliminary analyses utilizing immunohistochemistry techniques are underway to compare the presence of lymphocytes within the mammaries throughout lactation. Lymphocyte presence can then be correlated with gene expression patterns. These investigations have the potential to impact the understanding of the role lactation plays in neonatal immune development from an evolutionary perspective.

Thurs June 23rd, 11.20: Development of Immunity

Developmental exposure to pollutants from agriculture and unconventional oil and gas extraction alters metamorphosis and innate antiviral immunity in the amphibian *Xenopus*

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Non-targeted populations, including humans, are increasingly exposed to a large number of water pollutants associated with agriculture and unconventional oil and gas extraction (UOG). These pollutants are detectable in most aquatic habitats, but their long-term effects at environmental relevant levels on host antiviral immunity remains unclear. We have established the amphibian *Xenopus* and the ranavirus Frog Virus 3 (FV3) as a reliable experimental platform for evaluating the effects of common waterborne pollutants, such as the insecticide carbaryl and a mixture of 23 UOG chemicals with endocrine disrupting activity on the development of amphibian antiviral immunity. *Xenopus* tadpoles were exposed to either carbaryl or an equimass amount of UOG chemicals (0.1, 1.0 and 10 μg/L) for 3 weeks, and then infected with FV3 at tadpole stages or after metamorphosis. Both types of pollutants significantly altered tadpole innate antiviral immune response, as evidenced by decreased TNF-α, IL-1β, Type I IFN and Mx1 gene expression. Exposure to UOG chemicals also increased susceptibility to FV3 as indicated by a substantial increase in viral load, as well as changes in weight gain and time for metamorphosis completion. Notably, deregulated expression for most of these innate immune genes persisted after metamorphosis. These findings suggest that agriculture- and UOG-associated water pollutants at low but ecologically–relevant doses have the potential to induce long term alterations of host-pathogen interactions and effective antiviral immunity.

Thurs June 23rd, 11.40: Development of Immunity

AMD3100-INDUCED LEUKOCYTE MOBILIZATION IN *LEUCORAJA ERINACEA*

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In the extensive history of bone marrow transplantation, understanding donor stem cell activation and mobilization of leukocytes into the bloodstream has been of significant interest to investigators trying to understand the mechanisms controlling hematopoiesis. Elasmobranchs (sharks, skates and rays) may serve a unique role to this end. These cartilaginous fish lack the endosteal niche present in mammals, possessing instead specialized hematopoietic organs (the Leydig and epigonal organs), similar in function to mammalian bone marrow (leukocyte production), but uniquely composed of only the vascular niche. It is within these tissues of elasmobranchs that hematopoietic stem and progenitor cells (HSPCs) are maintained and activated to produce the full array of innate and adaptive leukocyte types. Many molecular and cellular interactions modulate hematopoiesis, but the chemokine receptor-ligand pair, CXCR4-CXCL12, is known to play a critical role in homing of HSPCs for cellular transplant engraftment, as well as maintenance of homeostasis in the bone marrow. Thus, inhibiting this connection with mobilizing agents, such as AMD3100, a clinically-utilized CXCR4 antagonist, induces mobilization of HSPCs. Our recent studies have identified, annotated, and assessed expression of CXCR4 and CXCL12 in the skate (*Leucoraja erinacea*) using genomic and transcriptomic sequence information from the North East Cyberinfrastructure Consortium, available at Skatebase.org. In addition, *L. erinacea* treated with AMD3100 exhibited significant leukocyte mobilization from the epigonal organ, as assessed via serological and immunohistochemical staining methods. Therefore, regarding pre-clinical protocols for both hematological and vascular diseases, important implications exist for the *L. erinacea* model in transplantation physiology.

Thurs June 23rd, 13.00: Genomics and Transcriptomics

Perturbation of immune state in the gut lumen induces system-wide cellular and transcriptional changes in the sea urchin larva

Katherine M. Buckley, Eric C.H. Ho and Jonathan P. Rast

The purple sea urchin (*Strongylocentrotus purpuratus*) genome sequence encodes a complex repertoire of genes encoding innate immune recognition proteins and homologs of important vertebrate immune regulatory factors. To characterize how this immune system is deployed within an experimentally tractable, intact animal, we investigate the immune capability of the larval stage. Sea urchin larvae are morphologically simple and transparent, which provides an organism-wide model to view immune response at cellular resolution. Seawater exposure to certain bacteria induces a robust larval cellular immune response that includes immune cell migration to the gut, changes in cell motility, and an increase in cell:cell interactions. Bacteria that accumulate in the gut later invade the blastocoel, where they are cleared by phagocytic and granular immune cells that express the immune effectors *185/333*. Coincident with this cellular response, next-generation sequencing indicates that much of the genome is transcriptionally regulated. The most strongly upregulated transcripts include a small family of IL17 homologs, which are rapidly upregulated in response to bacterial detection. Expression of these cytokines is restricted to the gut epithelium, and perturbation of IL17 signaling affects the expression of downstream immune signaling and effector genes. Expression levels also change for genes encoding transcription factors (*e.g.*, NfκB, Irf), signaling molecules (*e.g.*, TNF, Mif) and pattern recognition receptors (*e.g.*, PGRPs). The complexity of this coordinated inflammatory program within the simple larval morphology provides a system in which to characterize processes that direct both aspects of the echinoderm-specific immune response as well as those that are shared with other deuterostomes.

Thurs June 23rd, 13.20: Genomics and Transcriptomics

DIVERSITY IN A PURPLE SEA URCHIN LARVAL MODEL OF MICROBIAL COLONIZATION AND IMMUNITY

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The purple sea urchin (*Strongylocentrotus purpuratus*) is a marine deuterostome that develops indirectly via a swimming, feeding larval stage. We recently described multiple larval immune cell types and characterized their functions in infection with *Vibrio diazotrophicus*. Exposure to environmental microbes modulates this response compared to larvae raised in uninoculated seawater. To investigate the influence of microbial colonization on immune system development and function, we isolated culturable, specifically associated bacteria from laboratory and ocean raised larvae, profiled these communities by 16S rRNA gene sequencing, and observed isolate-inoculated larvae using 3D, time lapse, and fluorescent imaging. We cultured larva associated *Gammaproteobacteria* and *Bacteroidetes* of the genera *Vibrio*, *Pseudoalteromonas*, *Colwellia*, and *Polaribacter*, consistent with reported bacterial diversity in adult sea urchins. *Pseudoalteromonas* and *Polaribacter* sp. form relatively long term associations with larvae after inducing a mild immune response. The larval microbiota also includes an opportunistic and potentially lethal strain of *Vibrio lentus*, as determined by 16S rRNA, *rpoD*, and *toxR* multilocus sequence typing. Fluorescent imaging experiments suggest that residents are normally present in the gut lumen, and can invade the body cavity through either gut or skin epithelia at sufficient concentrations. Neither virulence nor residence time appear to be associated with *in vitro* growth or biofilm production. Current efforts include high-throughput 16S rRNA sequencing of laboratory and ocean raised eggs, embryos, and larvae, as well as characterizing (1) differential immune gene expression with different exposures, (2) microbiota-immune interactions during colonization, and (3) how resident microbiota influence immune response.

Thurs June 23rd, 13.40: Genomics and Transcriptomics

RNA-Seq: A Powerful Tool for Immune Pathway Discovery in Non-Model Organisms

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Next-generation sequencing (NGS) has revolutionized our ability to investigate complex physiological processes like immunology in non-model organisms in a way that was previously only been available for model organisms. Transcriptomic studies using RNA-Seq can discover, and measure the expression of, hundreds of thousands of transcripts from any species or tissue of interest. The combination of bioinformatic algorithms and sequencing depth, enables us to gain significant insight into the molecular processes mediating physiological changes. However, effective and accurate gene annotation is critical to drawing the proper conclusions from the massive amount of data that NGS provides. It is in this area that non-model organism investigations lag behind. Typically, less than 50% of annotated transcripts share orthology with proteins in the NCBI nr or Swiss-Prot databases. In most cases these are highly conserved metabolic or regulatory genes that have a secondary or tertiary role, if any at all in, immune response.

Immune genes have evolved rapidly over time, facilitating speciation and adaption to highly divergent ecological niches. As such, complete and proper annotation of immune genes from invertebrates, and crustaceans in particular, has been challenging. The purpose of this presentation is to highlight some of the challenges and successes when using RNA-Seq to expand our knowledge of the critical factors and signaling pathways comprising an invertebrate organism's immune system. Examples will be illustrated using the American lobster (*Homarus americanus*) as a model. The basics of RNA-Seq and the availability of analytical methods designed for those without computer programming expertise will be presented.

Thurs June 23rd, 14.00: Genomics and Transcriptomics

Influence of microbiome during olfactory epithelium development

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Olfaction is one of the most ancient and conserved systems among terrestrial and aquatic vertebrates. In the olfactory epithelium, the olfactory sensory neurons (OSNs) detect external stimuli (odors) and translate them into appropriate responses (chemical signals). Individual OSN are also constantly exposed to microbial signals produced by pathogens as well as the nasal microbiota. The microbiota is known to control most of the host's physiological functions, however, its potential role in the regulation of olfactory systems is unknown. We hypothesized that the microbiota plays a conserved role in the control of olfactory systems in vertebrates. To investigate our hypothesis, we used the olfactory organ of germfree and conventionalized zebrafish and mouse. Oligomicroarray transcriptomic analysis revealed that germfree animals express very lower levels of olfactory and vomeronasal receptors compared to their conventionalized counterparts. Additionally, expression of calcium related genes, tight junction genes, transcription factors and immune genes was impaired in the absence of the microbiota. These modifications suggested an abnormal development of the olfactory organ in germfree individuals. The latter was consistent with ultrastructural changes observed by electron microscopy as well as a different expression of G-coupled proteins observed by confocal microscopy in germfree compared to conventionalized zebrafish. Furthermore, the nasal commensal bacterium, *Staphylococcus sp*., was sufficient to drive the differentiation of the *Odora* olfactory sensory neuron cells. Altogether these results prove the fundamental and conserved role of the microbiome in the development and regulation of olfactory systems in vertebrates.

Thurs June 23rd, 14.20: Genomics and Transcriptomics

Development of a Multiplex Nucleic Acid Assay for Salmon Immunological Assessment

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Immunological or other biological response assessment is currently limited to two levels of throughput**;** (1) **s**ingle target platforms such as qPCR or ELISA,(2) RNA-Sequencing or microarray platforms. The Luminex® X-MAP® (X-MAP) technology allows for simultaneous quantification and monitoring of multiple biomarkers from the same sample, thereby providing a medium-range platform for rapid and sensitive testing. A suite of nucleic acid markers frequently associated with viral, bacterial or ectoparasitic infections in Atlantic salmon (*Salmo salar*), were chosen to determine their sensitivity and dynamic range for inclusion in this custom multiplex assay. The goal of this study was to provide a single test for use in research or diagnostic laboratories that could be used to assess Atlantic salmon infection status and response to vaccination as well as other important production characteristics related to health.
Head kidneys from Atlantic salmon smolts exposed to either infectious salmon anemia virus (ISAv), the parasitic copepod, *Lepeophtheirus salmonis,* or both were analyzed on qPCR and X-MAP platforms, using a suite of anti-parasitic/anti-viral gene markers along with commonly used reference genes. Analysis was completed on individual animals and showed excellent agreement with the calibrated normalized relative quantity (CNRQ) by qPCR for mean fold differences between infected and control animals; interleukin-1β showed 5.1-fold increase by qPCR and 4.9-fold increase by X-MAP; matrix metalloprotease-9 showed 1.3-fold increase by qPCR and 1.2-fold increase by X-MAP. In addition, different concentrations of template (salmon DNase-treated RNA) were compared (50-400 ng) across control and infected animals on the X-MAP platform, and the 100 ng assay was found to achieve the optimal results with respect to reproducibility and sensitivity, supporting efficiencies in work-flow, reagents and sample conservation for this technology. The Atlantic salmon custom multiplex assay is currently being expanded to include important T- and B-cell markers to analyze vaccination responsive genes. The assay's performance with respect to the different anti-viral responses will also be discussed.

Thurs June 23rd, 14.40: Genomics and Transcriptomics

DEEP SEQUENCING OF *B. TAURUS* IMMUNOGLOBULIN HEAVY CHAIN REPERTOIRE

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Ruminants have a low number of germline immunoglobulin heavy chain gene segments (IgH) resulting in low combinatorial diversity, thus the repertoire potential of antibody responses. Recent genomic restructuring revealed the IgH locus of the ruminant species *Bos taurus* contains up to 12 functional VH segments, subdivided into three polymorphic families, 23 functional DH segments, and four functional JH segments. However ruminant immune systems utilize an innovative mechanism to expand this limited repertoire, whereby naïve B cells undergo somatic hypermutation in the periphery before cognate antigen activation. Additionally a VH-DH rearrangement of segments unique to *B. taurus* encodes a CDR3 of greater than 40 amino acid residues, known as an ultralong CDR3 (ULCDR3) receptor. The ULCDR3 crystal structure was fond to possess a knob, formed through uniquely folded disulfide bonds induced by naïve mutations of germline residues to cysteines, held at the end of a protruding stalk which plays a primary role in antigen specificity. A targeted deep sequencing approach of peripheral blood lymphocytes was implemented to assess the diversification potential of the IgH repertoire of *B. taurus*. Deep sequencing yielded full transcripts for 6093 unique clones with an ULCDR3 population of ten percent. Tissue specific deep sequencing revealed CDR3 length varies significantly amongst tissue with the Peyer's patch housing the longest average CDR3 length. This was directly correlated to tissue differences in ULCDR3 population. Since recombination events occur in secondary lymphoid tissue of fetal calves it remains unclear whether tissue differences in CDR3 length are attributed to preferential recombination in a resident tissue or a chemotactic signal directing ULCDR3 B cells.

Thurs June 23rd, 15.20: Innate immunity: Antiviral and PRRS

ATYPICAL NATURAL KILLER T-CELL RECEPTOR RECOGNITION OF CD1d-LIPID ANTIGEN

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The T lymphocytes repertoire is divided into two major lineages, αβ and γδ T-cells, which are defined by their T-cell receptor (TCR) gene-segment usage. To date, most of the groundbreaking discoveries on human CD1d-α-galactosylceramide (α-Galcer) reactive T-cells have focussed on the type I Natural Killer T cells (NKT) subset that express an invariant TCR α-chain (TRAV10-TRAJ18) which pairs with a β-chain (TRBV25-1). The structural basis for the molecular recognition of CD1d-lipid antigen by type I NKT cells is also now well established [1, 2]. However, despite their biological importance, the extent of the diversity of the NKT cell repertoire is still unclear. Here, we identified a TRAV10-TRAJ18-TRBV25-1$^-$ population of human CD1d-α-Galcer reactive NKT cell subsets that expressed atypical αβ TCRs [3], γδ TCRs [4] and unusual δ/αβ TCRs [5]. Furthermore, we provided the molecular mechanisms that underpin the recognition of lipid antigen by these distinct subsets of CD1d-restricted T-cells. Our findings highlight the emergence of diverse populations of NKT TCRs that exhibit different binding mode, and provide a greater scope for diverse glycolipid antigens recognition by CD1d-restricted T-cells subsets. Ultimately, our study radically reshapes our understanding of NKT TCR molecular recognition.

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[3] Le Nours J., *et al*. *Nature Commun.*, 2016.
[4] Uldrich A. U. & Le Nours J., *et al. Nature Immunol.*, 2013.
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Thurs June 23rd, 15.40: Innate immunity: Antiviral and PRRS

DSRNA SENSING IN FISH: USING CHSE-214 AS A MODEL FOR STUDYING CLASS A SCAVENGER RECEPTORS

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When infecting cells, most viruses produce dsRNA molecules at some point in their life cycle. Class A scavenger receptors (SR-As) on the surface of animal cells bind these foreign molecules and bring them into the cell where dsRNA triggers an innate immune response, enabling the cell to protect itself from an impeding virus infection. Very few cells are unable to bind extracellular dsRNA via SR-As, thus cell-based assays for studying SR-A function and signaling have been limited. CHSE-214 cells are a promising model for scavenger receptor study as they have been shown to induce Mx protein as well as an antiviral state against IPNV, in response to transfected dsRNA but not to extracellular dsRNA; possibly due to a lack of functional scavenger receptors. Using antiviral and cytopathic effect assays and qRT-PCR to look at the expression of interferon and interferon-stimulated gene transcripts, we have confirmed that CHSE-214 cells only respond to dsRNA transfected directly into the cell. Fluorescence microscopy has also shown that transfection of these cells with a hSR-AI-containing expression vector increases AcLDL uptake, a classical SR-A receptor ligand. To further investigate the feasibility of CHSE-214 functioning as a cell model for SR-A binding and signaling, rainbow trout SR-A sequences cloned into expression vectors will also be transfected into CHSE-214 cells to test their ability to respond to extracellular dsRNA.

Thurs June 23rd, 16.00: Innate immunity: Antiviral and PRRS

SpTransformer, a multitasking recombinant Sp185/333 protein from the purple sea urchin binds to multiple targets

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The purple sea urchin, *Strongylocentrotus purpuratus*, has a complex and sophisticated innate immune system that includes several large gene families. One of these is the *Sp185/333* gene family composed of ~50±10 (estimated) small genes that are tightly linked in clusters, share blocks of sequence called *elements* that are present in mosaic patterns, plus have a variety of repeats within the second exon. Each gene is surrounded by one or two types of short tandem repeats in an unusual genomic structure. These characteristics suggest that the regions harboring the *Sp185/333* genes may be unstable and may drive sequence diversification of the genes, which would be a benefit in anti-pathogen immunity.

The anti-pathogen activities of a recombinat Sp185/333 protein, called rSpTransformer (rSpTrf), binds specifically to *Vibrio* and yeast, but not to *Bacillus*. It also binds LPS, β-1,3-glucan, and flagellin with specificity and high affinity, but does not bind peptidoglycan. rSpTrf also binds phosphatidic acid (PA), deforms liposomes composed of 10% PA and induces budding, fusion, invagination and leakage. rSpTrf is intrinsically disordered but transforms to alpha helical in the presence of LPS or PA, suggesting "ShapeShifter" activities for binding lipids, sugars and proteins. Given that single sea urchins are capable of expressing up to 260 Sp185/333 protein variants, if each one has a range of overlapping binding activities that target simultaneously multiple PAMPs, this may provide highly effective and flexible host protection against a broad array of potential pathogens encountered in the marine environment.

Thurs June 23rd, 16.20: Innate immunity: Antiviral and PRRS

UNDERSTANDING THE INNATE ANTIVIRAL IMMUNE RESPONSE TO FROG VIRUS 3 (FV3)

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Ranavirus infections are becoming increasingly prevalent worldwide and have been implicated in numerous species die-offs across the globe. Frog virus 3 (FV3) is the type-species of the genus, yet its virus-host interactions remain poorly understood. Chief among these interactions is the type 1 interferon (IFN) response. Following infection, type 1 IFNs trigger an "antiviral state" in the host cell via the production of numerous interferon-stimulated genes (ISGs) that act to inhibit virus replication in various ways. This study utilizes two rainbow trout cell lines, RTgutGC (epithelial; intestinal origin) and RTG-2 (fibroblastic; gonadal origin), previously shown to differ in susceptibility to FV3 and, as such, serve as an excellent model to study innate anti-FV3 responses. Cell viability assays were performed to quantify differences in the extent of cell death over time. RTG-2 appears to be more susceptible than RTgutGC, exhibiting greater cell death at a lower virus titre. The mechanism of cell death was investigated via DAPI staining and DNA laddering to observe nuclear fragmentation, a hallmark of apoptosis. Both cell lines appear to undergo apoptosis, which is regarded as an important antiviral defense mechanism. Real-time RT-PCR was performed to investigate differences in IFN, ISG and viral transcript expression between the two cell lines. Surprisingly, IFN and ISG induction was not observed in either cell line, but viral transcripts appear to be drastically higher in RTG-2 than RTgutGC, indicating a higher level of infection. Thus, the antiviral mechanisms controlling infection in RTGutGC remain to be elucidated, yet appear to be IFN-independent.

Thurs June 23rd, 16.40: Innate immunity: Antiviral and PRRS

LOBSTERS IN HOT WATER: IMPACT OF TEMPERATURE ON THE AMERICAN LOBSTER'S MOLECULAR IMMUNE RESPONSE TO WSSV

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Many marine invertebrates exhibit temperature-dependent immune responses. Variable water temperatures pose biological challenges, as processes such as development, growth, and reproduction are temperature-dependent. American lobster inhabit ocean waters that fluctuate in temperature between 0 ^{o}C and 20 ^{o}C. The full impact of these temperature variations on the immune response of *Homarus americanus* is unknown. White Spot Syndrome Virus (WSSV) is currently one of the largest impediments to the shrimp aquaculture industry. The World Organization for Animal Health (OIE) lists WSSV as a notifiable disease with the potential to infect all crustacean decapods. Earlier work has demonstrated that upon intramuscular injection at 20 ^{o}C, WSSV infects and replicates within the atypical lobster host. The present study utilized a WSSV injection challenge model, to explore the molecular immune response of American lobster held at 4 temperatures (10 ^{o}C, 15 ^{o}C, 17.5 ^{o}C, 20 ^{o}C). A lobster specific microarray containing 14,592 genes, was used to monitor transcriptomic changes in hepatopancreas mRNA, during viral infection at the different temperatures. A total of 383 genes were significantly differentiated across the temperature groups. Genes of interest included crustacean immune-related genes such as crustin, thioredoxin, serum amyloid A, and prostaglandin E synthase 2. RT-qPCR was used for gene expression confirmation. Information from this study will help to characterize temperature-dependent immune function in American lobster.

Thurs June 23rd, 17.00: Innate immunity: Antiviral and PRRS

IN VITRO TRANSCRIBED DSRNA AS A SURROGATE FOR NATIVE DSRNA IN RAINBOW TROUT CELLS

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Viruses produce double-stranded (ds)RNA during their replicative life cycles; these dsRNA molecules are known as native dsRNA. DsRNA molecules are potent immunomodulators that induce innate immune responses; extracellular dsRNA molecules in both mammals and fish are brought into host cells via class A scavenger receptors (SRAs). Current research focuses on the synthetic, viral dsRNA mimic, poly I:C; there are currently no studies using native dsRNA as stimulants for fish cells. As isolating native dsRNA can prove time-consuming and inefficient, in vitro transcribed dsRNA (IV-dsRNA) may prove to be an excellent candidate for a novel immunostimulant, as it is easy to make and more biologically relevant compared with poly I:C, assuming it induces host responses similarly to native dsRNA. One strength for IV-dsRNA is that it can be constructed to have the natural sequence variation and defined length that poly I:C lacks. The current study compares both IV-dsRNA and native dsRNA-induced innate immune responses. Entry mechanisms for native and IV-dsRNA were both SRA-mediated in the rainbow trout gut cell line (RTgutGC). As is seen in mammals, extracellular dsRNA entry involves clathrin-mediated endocytosis, as determined using the inhibitor chlorpromazine. qRT-PCR and an antiviral assay were used to compare the innate immune responses of a rainbow trout gonadal cell line (RTG-2) to the molecules; both induced similar responses. A more thorough understanding of how host cells respond to biologically relevant dsRNA molecules and possible surrogates has applications in further studies as well as adjuvant and antiviral therapy design.

Thurs June 23rd, 17.20: Innate immunity: Antiviral and PRRS

Characterisation of an Atlantic Salmon Heart Endothelial (ASHe) Cell Line and Evaluating its Ability to Support Fish Virus Infection

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Studying the Atlantic salmon heart is important for understanding fish cardiovascular diseases, which are increasingly being noticed in aquaculture. As an aid for studying the Atlantic salmon heart, an Atlantic salmon heart endothelial cell line, ASHe, was developed and characterized for general properties, endothelial cell features, and ability to support fish virus replication. AHSe cells grew well in 10% FBS/L15, had preference for collagen coated surfaces and were negative for senescence-associated β galactosidase. ASHe displayed several important endothelial characteristics including a cobblestone morphology, circumferential actin localization, expression of von Willebrand factor (vWF) and tight junction proteins, capacity to form capillary-like structures, and responsiveness to lysophosphatidic acid (LPA). At 5 and 25 μM, LPA reduced wound healing and capillary formation but increased ASHe cell metabolism and colony formation. The response of ASHe cells to LPA was conflicting but is similar to what has been described in the literature for mammalian endothelial cells. Therefore, this cell line could be a useful model for future comparative studies on the cell biology of piscine and mammalian cardiovascular systems. When ASHe cells were exposed to four fish viruses – infectious pancreatic necrosis virus (IPNV), chum salmon reovirus (CSV) and viral hemorrhagic septicemia virus (VHSV type IVa and IVb) – all four viruses replicated with VHSV IVa producing the highest viral titre. Therefore, in addition, ASHe should be valuable for studying cardiovascular diseases of viral origin in fish.