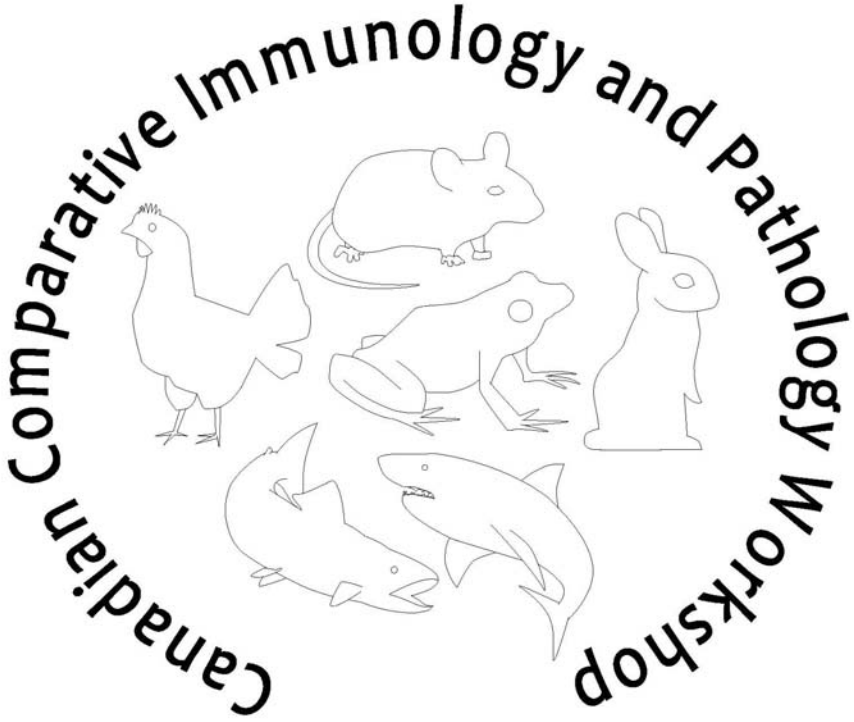
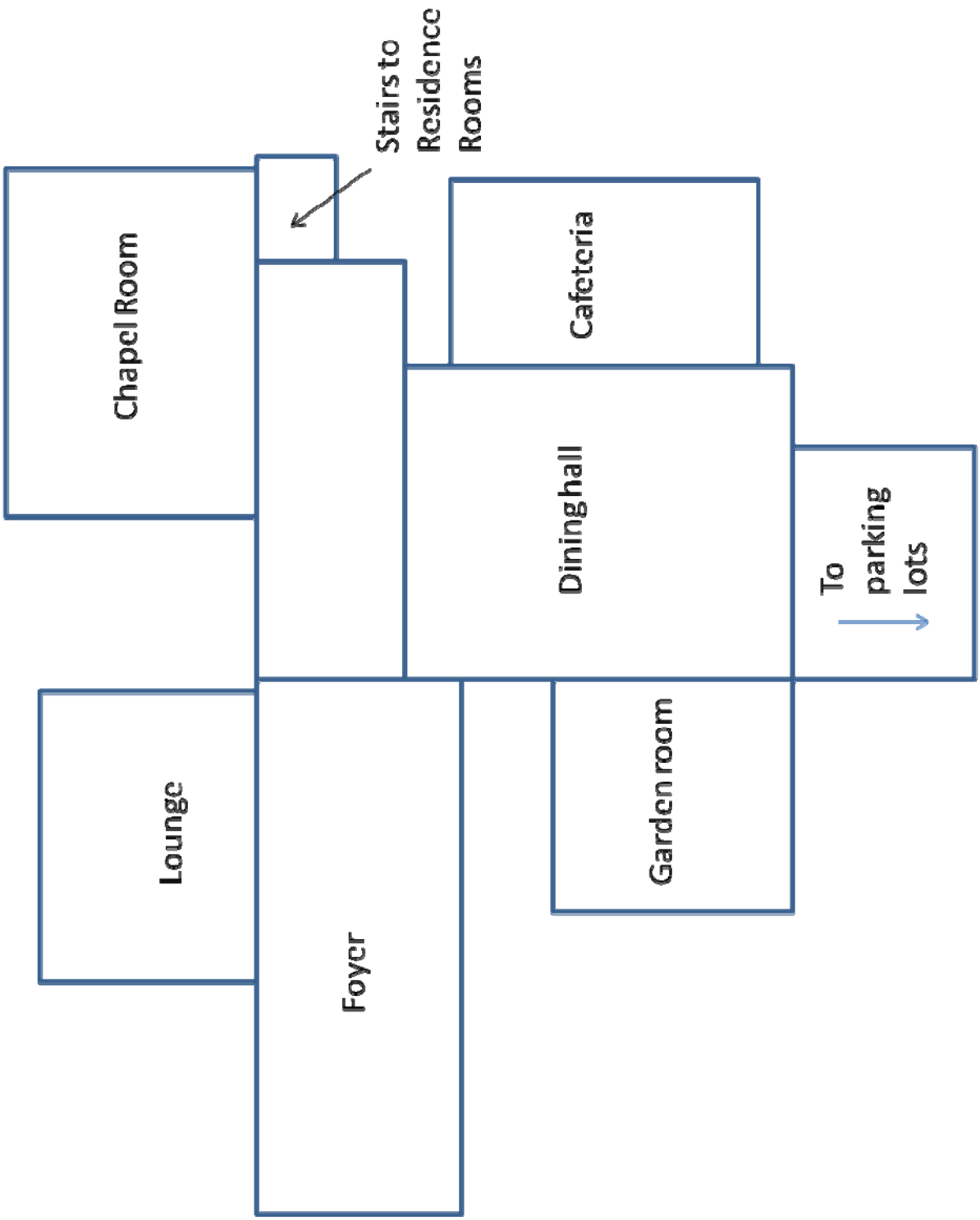


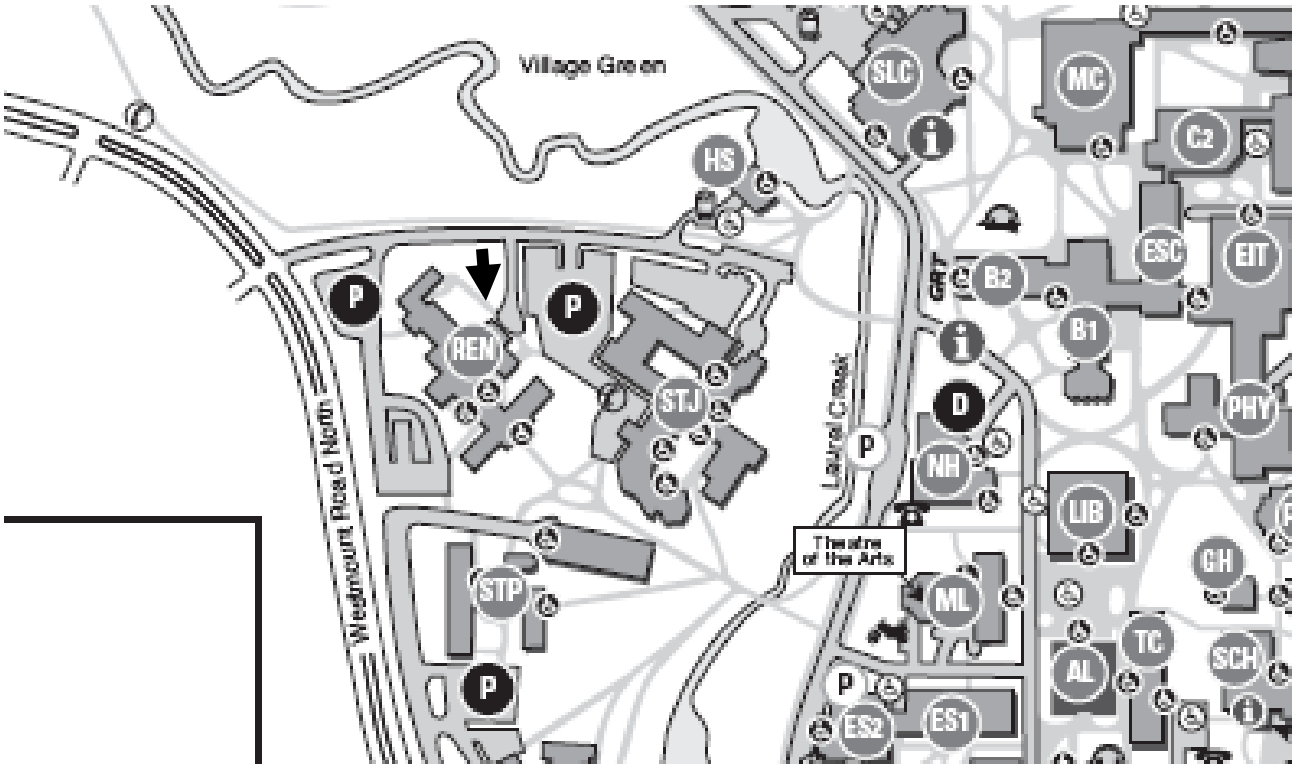
May 26th to 28th, 2010
Renison University College
University of Waterloo

MAPS

Room Map



Area Map



REN = Rension College
Arrow = Entrance
P = Parking lots (The two on either side of Rension a $4 per entry, coin only)

Schedule at a glance

Time	Weds May 26th	Thurs May 27th	Fri May 28th	Sat May 29th
8.00 - 9.00		Breakfast: Dining Hall	Breakfast: Dining Hall	
9.00- 10.00		Welcome + Dave Speare: Plenary	Martin Flajnik: Plenary	Breakfast
10.00 - 10.30		Coffee: Foyer	Coffee: Foyer	Departure
10.30 - 10.50		Kathy Magor	Kevin Robinson	
10.50-11.10		Patrick Hanington	Douglas Hodgins	
11.10-11.30		Richelle Monaghan	Leandro Becker	
11.30-11.50		Ayoola Oladiran	Xinzhong Wu	
noon -1.00		Lunch: Dining Hall	Lunch: Dining Hall	
1.00-1.20		Katherine Buckley	Dan Barreda	
1.20-1.40		John Lumsden	Leon Grayfer	
1.40-2.00		Ana Goyos	Jacques Robert	
2.00-2.20		Ben Montgomery	Marije Booman	
2.20-2.40		Hristina Nedelkovska	Hillary Vanderven	
2.40-3.00		Jonathan Rast	Cynthia Tang	
3.00-3.30		Coffee: Foyer	Coffee: Foyer	
3.30-3.50	Arrival/Registration: Foyer	Ayisa Chida	PI meeting to discuss future workshops	
3.50-4.10		Brad Magor		
4.10-4.30		Cynthia Messier-Solek		
4.30-4.50		Motoshige Yasuike		
4.50-5.10		Erick Garcia		
5.30-6.30		Dinner: Dining Hall	Banquet (drinks start at 6pm):	
7.00 10.00	Welcome Wine & Cheese: Garden Room	Poster session: Foyer cash bar/snacks	Meal: Garden Room/Dining Hall	

Detailed Schedule:

Wednesday May 26th, 2010

3.00pm: Registration starts in the Foyer

7.00pm: Welcome wine and cheese in the Garden Room

Thursday May 27th, 2010

8.00am Breakfast – Dining Hall/Garden Room

9.00am Welcome – Chapel Room

9.05am Comparative Pathology Plenary Lecture - David Speare**: Fish Health "Under the Microscope"**

10.00am Coffee Break: Foyer

10.30am Session 1: Pathogens and Parasites Chapel Room – Moderator Kathy Magor

10.30-10.50 Kathy Magor: *Association of RIG-I with innate immunity of ducks to influenza*

10.50-11.10 Patrick Hanington: Characterization of the defense response generate by *Biomphalaria glabrata* following challenge with the trematode parasites *Echinostoma paraensei* and *Schistosoma mansoni*

11.10-11.30 Richelle Monaghan: Growth of an insect derived microsporidian, *Anncaliia algerae* (*Nosema*, *Brachiola*) in rainbow trout cell lines

11.30-11.50 Ayoola Oladiran: Immune Evasion Mechanisms of *Trypanosoma carassii*

12.00-1.00pm Lunch – Dining Hall/Garden Room

1.00pm Session 2: Receptors Chapel Room – Moderator Jonathan Rast

1.00-1.20 Kathleen Buckley: Complex and dynamic multigene families of innate receptors suggest a new form of animal immunity in the sea urchin

1.20-1.40 John Lumsden: Plasma pattern recognition receptors of rainbow trout (*Onchorhynchus mykiss*)

1.40-2.00 Ana Goyos: Distinct Nonclassical MHC Class Ib Gene Lineages are Conserved in Divergent Amphibian Species

2.00-2.20: Ben Montgomery: Characterization of innate immunoregulatory receptors in the channel catfish (*Ictalurus punctatus)*

2.20-2.40: Hristina Nedelovska: Investigating the roles of hsp72 and hsc73 in MHC class I-mediated immune surveillance in the frog *Xenopus laevis*

2.40-3.00: Jonathan Rast: A simple model for systems-level innate immunity

3.00-3-30pm Coffee Break: Foyer

Thursday May 27th, 2010

3.30pm Session 3: Evolution and Development of Immunity – Moderator Brad Magor

3.30-3.50 Asiya Chida: Phylogeny and ontogeny of CD4, CD8α and CD8β T cell co-receptor homologs in two amphibian species: *Silurana tropicalis* and *Xenopus laevis*

3.50-4.10 Brad Magor: The cellular context of AID expressing cells in fish lymphoid tissue

4.10-4.30 Cynthia Messier-Solek: Gata and bHLH factors form a conserved regulatory circuit for deuterostome immunocyte development

4.30-4.50 Motoshige Yasuike: Evolution of duplicated *IgH* loci in Atlantic salmon (*Salmo salar*)

4.50-5.10 Erick García-García: Characterization of lipid rafts in fish immune cells

5.30-6.30 Dinner – Dining Hall/Garden Room

7.00-10.00 Poster Session: Foyer

Friday May 28th, 2010

8.00am Breakfast – Dining Hall/Garden Room

9.00am Comparative Immunology Plenary Lecture – Martin Flajnik**: Evolution of Antigen Receptors**

10.00am Coffee Break: Foyer

10.30am Session 4: Environment and Husbandry Chapel Room – Moderator Brian Dixon

10.30-10.50 Kevin Robinson: Comparing the Immune Response of the Brown Bullhead Catfish (*Ictularus nebulosus)* from Clean and Contaminated Sites along the Detroit River

10.50-11.10 Douglas Hodgins: Vaccination of Neonatal Calves: Obstacles and Opportunities

11.10-11.30 Leandro Becker: Breeding strategy and early rearing environment affect the immune response in Chinook salmon (*Oncorhynchus tshawytscha*)

11.30-11.50 Xinzhong Wu: Interaction between pathogen rickettsia-like organism and its host, oyster *Crassostrea ariakensis*

12.00-1.00pm Lunch – Dining Hall/Garden Room

1.00pm Session 5: Macrophage Function and Immune Gene Expression Chapel Room – Moderator Daniel Barreda

1.00-1.20 Daniel Barreda: Macrophage activation differentially modulates binding, internalization and downstream killing responses during phagocytosis

1.20-1.40 Leon Grayfer: Assessment of macrophage antimicrobial responses induced by type II interferons of the goldfish (*Carassius auratus* L.)

1.40-2.00 Jacques Robert: Critical role of macrophages in amphibian immune surveillance

2.00-2.20 Marije Booman: Development of an Atlantic cod (*Gadus morhua*) oligonucleotide microarray and its validation using a study of cod spleen global gene expression responses to stimulation with formalin-killed atypical *Aeromonas salmonicida*

2.20-2.40 Hillary Vanderven: Upregulation of immune genes in duck lung and intestine during high and low pathogenic avian influenza infection

2.40-3.00 Cynthia Tang: Functional Characterization of the Rainbow Trout (*Oncorhynchus mykiss*) Chemokine, CK-2

3.00-3-30pm Coffee Break: Foyer

3.30pm–4.30pm PI meeting to discuss future workshops - Chapel Room

6.00pm Banquet Drinks

7.00pm Banquet and Awards Presentation

Plenary Talk Thursday May 27th: Comparative Pathology: Fish Health "Under the Microscope".

David Speare
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Advances in fish health have come about through many participating "ologies" and scientific approaches, and there is almost no limit to the number of problems still needing to be solved particularly when we consider the varieties of environmental settings, range of species, and – at least in the case of aquaculture – client expectations. Pathology, as a discipline, seeks to gain further understanding of disease processes through examination of host responses reflected through tissue changes. Comparative pathology examines differences and similarities between species, often using the relatively well-studied human system as a starting point. Can we use this approach to better understand the pathogenesis of infectious and non-infectious fish diseases? In turn, does comparative pathology provide any unique insights when it comes to elucidating treatment or management solutions? Examples, based on selected disease conditions from Canadian aquaculture, will be used to explore these questions.

Plenary Talk Friday May 28th: Evolution of Antigen Receptors

Martin Flajnik
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The cartilaginous fish have been critical to our understanding of the evolution of adaptive immunity. Sharks have been known to possess antibodies since the mid-60s when they were discovered by Marchalonis and Clem. In the mid-80s Litman showed that shark and skate Ig heavy and light chain genes of all types are found in the "cluster configuration," and suggested that such an organization would provide flexibility and opportunities for the emergence of novel types of antigen receptors. Building on these seminal discoveries (but against conventional wisdom) our work has shown that the shark antibody response indeed matures via mutation and selection as well as a "switch" from pentameric to monomeric forms. Regarding light chains, it is now clear that that there were four major groups at the inception of the adaptive immune system, with lambda and kappa being two of the groups; furthermore, the isotype IgD now has been shown to be as old as IgM, but exceedingly more plastic over evolutionary time. Consistent with Litman's original idea, novel forms of antibodies have also emerged, including: specialized "germline-joined" Igs expressed early in development; antibodies with single Vs that do not associate with light chains; and new forms of the TCR that blur the distinction between antibodies and TCRs. In summary, our work has helped to show what is primordial in adaptive immunity as well as the emergence of molecules/mechanisms unique to cartilaginous fish.

Thursday May 27th, 10.30-12.00 Pathogens and Parasites

Association of RIG-I with innate immunity of ducks to influenza

Megan Barber[a], Jerry Aldridge[b], Robert Webster[b] and Katharine Magor[a]*
[a]Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, kmagor@ualberta.ca; and [b]St. Jude Children's Research Hospital,

Ducks and wild waterfowl perpetuate all strains of influenza viruses in nature. In their natural host, influenza viruses typically cause asymptomatic infection and little pathology. Ducks are often resistant to influenza viruses capable of killing chickens. Here, we show that the influenza virus sensor, RIG-I, is present in ducks and plays a role in clearing an influenza infection. We show evidence suggesting that RIG-I may be absent in chickens, providing a plausible explanation for their increased susceptibility to influenza viruses compared with ducks. RIG-I detects RNA ligands derived from uncapped viral transcripts and initiates the interferon response. In this study, we show that the chicken embryonic fibroblast cell line, DF-1, cannot respond to a RIG-I ligand. However, transfection of duck RIG-I into DF-1 cells rescues the detection of ligand and induces interferon-beta promoter activity. Additionally, DF-1 cells expressing duck RIG-I have an augmented interferon response resulting in decreased influenza replication after challenge with either low or highly pathogenic avian influenza virus. Implicating RIG-I in the antiviral response to an infection in vivo, we found that RIG-I expression is induced 200 fold, early in an innate immune response in ducks challenged with the H5N1 virus A/Vietnam/1203/04. Finding this natural disease resistance gene in ducks opens the possibility of increasing influenza resistance through creation of a transgenic chicken.

Characterization of the defense response generate by *Biomphalaria glabrata* following challenge with the trematode parasites *Echinostoma paraensei* and *Schistosoma mansoni*

Patrick C. Hanington*, Michelle A. Forys, Coen M. Adema and Eric S. Loker
Center for Evolutionary and Theoretical Immunology, Department of Biology, University of New Mexico, Albuquerque, NM, 87131, pch1@unm.edu

Transcriptional analysis of *Biomphalaria glabrata* snails following challenge with the digenetic trematodes *Schistosoma mansoni* and *Echinostoma paraensei* resulted in discovery of a number of transcripts associated with snail resistance to infection. Comparison of the transcriptional profiles expressed by snails resistant to infection because of age/size, strain (BS-90/M-line) or prior exposure to homologous parasites (acquired resistance) yielded a pattern in which some transcripts were commonly up regulated in resistant snails and down regulated in snails that were infected. From this comparison a number of resistance-associated transcripts were identified, some being commonly up regulated in all three types of resistant snails. One molecule displaying this type of pattern was fibrinogen related protein 3 (FREP3). The common recurrence of FREP3 in all of our transcriptional studies of snail resistance, and the sequence heterogeneity that arises in FREP3 molecules due to a high incidence of point mutation and putative gene conversion events, made FREP3 a high priority for further functional analysis. To identify possible sources of the increased FREP3 levels *in situ* hybridization studies were performed, in these studies newly produced hemocytes were co-labeled with BrdU and FREP3 suggesting that newly developed hemocytes are involved in the production of FREP3. Using an anti-FREP3 antibody we purified native FREP3 from *B. glabrata* plasma and used both FREP3 and the antibody to it to analyze FREP3 function. We have demonstrated that FREP3 is involved in recognition and binding of galactose sugars, and that it can act as an opsonin to enhance phagocytosis of bound targets. Preliminary RNA interference studies knocking down FREP3 expression partially abrogate the defense response of resistant snails to the parasite, allowing infection to occur. We hypothesize that FREP3 represents a critical defense molecule that has an important role in protecting the snail against trematode infection.

Growth of an insect derived microsporidian, *Anncaliia algerae* (*Nosema*, *Brachiola*) in rainbow trout cell lines

SR Monaghan[1,2*], RL Rumney[2], NC Bols[1], LEJ Lee[2]
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Microsporidia are intracellular parasites of fungal origin, of which *Anncaliia algerae* is one of over 1500 species described to date. Initially reported in *Anopheles* mosquito and believed to only infect insects, *A. algerae* has subsequently demonstrated to parasitize a number of insect and mammalian hosts. This growth is mirrored in the ability of the parasite to propagate in insect and mammalian cell cultures. Although fish feed on insect hosts of *A. algerae*, and are themselves hosts to over 150 microsporidia, *A. algerae* is not known to be a microsporidian parasite of fish. However, the cultivation of *A. algerae* in goldfish, zebrafish and fathead minnow derived cell lines at 27ºC demonstrates the first report of this parasite propagating in fish cells. Preliminary work also shows that *A. algerae* growth is supported in rainbow trout cell lines at temperatures of 21 to 22ºC. The rate of *A. algerae* growth at these lower temperatures appears to be inhibited by approximately 50 percent with sporont development at day 4 post-inoculation in contrast to day 2 when incubated at 27ºC. Additionally, *A. algerae* is readily phagocytized by the rainbow trout derived monocyte/macrophage cell line, RTS-11. Macrophages, by transporting phagocytized spores, are thought to play a role in disseminating microsporidiosis. Using phase contrast and fluorescence microscopy following staining with 1 µg/ml of 4',6-diamidino-2-phenylindole (DAPI), RTS-11 cells are observed to phagocytize an average of 9.8 *A. algerae* spores after 3 hours, and 10.8 and 15.3 spores after 6 and 12 hours, respectively. Although host or tissue tropisms may restrict growth of some microsporidia, this research demonstrates the rapid phagocytosis of *A. algerae* spores by RTS-11 and growth of the parasite in fish cell lines, including rainbow trout derived cell lines, indicating there may be a potential for *A. algerae* infection of fish under the appropriate conditions.

Immune Evasion Mechanisms of *Trypanosoma carassii*

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Trypanosoma carassii is a parasite of economically important fish species that is evolutionarily related to *T. brucei* and *T. cruzi*. *T. carassii* has evolved several strategies to circumvent host immune responses. We identified *T. carassii* HSP70 (Tcahsp70) and calreticulin (TcaCRT) as candidate antigens in excreted/secreted fraction and in membrane proteins of cultured parasites. We cloned and produced *T. carassii* hsp70 (rTcahsp70) and calreticulin (rTcaCRT), and generated a rabbit polyclonal antibody to the recombinant proteins. These antibodies recognize native proteins in ES products and parasite lysate. Recombinant hsp70 (rhsp70) activated goldfish macrophages and upregulated genes encoding pro-inflammatory cytokines and chemokines. Parasite hsp70 also upregulated the expression inducible nitric oxide synthase (iNOS) and induced a strong nitric oxide response of goldfish macrophages. Recombinant parasite calreticulin bound several molecules in host serum including the first complement component, C1q. The host C1q specifically interacted with rTcaCRT since the C1q-dependent lysis of sensitized sheep erythrocytes was inhibited by rTcaCRT. The incubation of parasites with rabbit anti-rTcaCRT affinity-purified IgG antibody indicated substantial CRT levels on the surface of trypanosomes, as well as internal structures of permeabilized organisms. Our findings suggest that Tcahsp70 may be secreted by the parasite to provoke a Th1-type immune response while TcaCRT may be used by the parasite to inhibit hosts' classical complement pathway. [Supported by NSERC, CANADA]

Thursday May 27th, 1.00-3.00 Receptors

Complex and dynamic multigene families of innate receptors suggest a new form of animal immunity in the sea urchin

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Toll-like receptors (TLR) serve a critical role in innate immunity by recognizing conserved microbial structures and initiating an immune response. These proteins consist of an intracellular Toll/Interleukin-1 receptor (TIR) signaling domain, and a variable ectodomain that interacts with pathogens. TLRs form small gene families in vertebrates (10 – 20 genes) and in *Drosophila* (9 genes). In contrast, the purple sea urchin genome encodes a large multigene family of >200 TLR genes. Most of the sea urchin TLR genes are structurally similar to vertebrate TLRs and form seven subfamilies based on phylogenetic analysis of the TIR sequence. Ectodomains of the remaining, more divergent, TLRs are either shortened or resemble that of *Drosophila* Toll. Levels of variation among subfamily members indicate regions of rapid diversification within the ectodomain that may be associated with ligand interaction or dimerization, and specific residues appear to be under positive selection. The high frequency of pseudogenes and similarity of genes within subfamilies suggest that the many TLR genes result from recent duplication that is consistent with birth and death mode of evolution. Many of the TLRs are arranged in small, homogeneous genomic clusters, while others are isolated. TLR clusters are typically composed of closely related members of a single subfamily, although some clusters are heterogeneous. TLR subfamilies are differentially expressed in larvae and coelomocytes, with the highest expression observed in the phagocytic coelomocytes. Significant TLR expression was not detected during embryogenesis. The multiplicity, diversity, and expression patterns of these receptors point to a novel immunological role for this gene family in the sea urchin. Similar TLR gene family expansions in two related species of sea urchin as well as a cephalochordate suggest that the vertebrate adaptive immune system most likely emerged from this type of complex immunity.

Plasma pattern recognition receptors of rainbow trout (*Onchorhynchus mykiss*)

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Two plasma lectins of rainbow trout were isolated by their ability to bid to several relevant microbes that cause disease in trout. Several gram-negative bacteria, lipopolysaccharide as well as chitin were targets of trout ladderlectin and intelectin. Intelectin (RTInt) was isolated for the first time from fish. A 975bp cDNA sequence obtained encoded a 325 amino acid secretory protein with homology to mouse and human intelectin. RTInt exhibited calcium-dependant binding to N-acetylglucosamine- and mannose-conjugated matrices and was present as at least five distinct 37 kDa isoforms on two-dimensional polyacrylamide gel electrophoresis (PAGE). Ladderlectin, a group VII mannose-binding C-type lectin, was found to have two cDNA sequences (RTLL-1 and -2), which had 92% sequence identity and encoded 173 and 187 amino acids, respectively. The genomic sequence of RTLL was found to encompass six exons and five introns, with exon 2 encoding 14 amino acids, which were exclusive to RTLL-2. Two-dimensional PAGE and western blots of whole plasma and of plasma proteins that bound both chitin and bacteria, demonstrated multiple electrophoretic isoforms of RTLL ranging in size from 16-18 kDa and isoelectric points between pH 4.9 and 6.3. Rabbit polyclonal antibodies were used for enzyme immunoassays to quantify both RTLL and RTInt. There was significant group and individual variation in plasma lectin concentration. Neither lectin was induced under conditions known to produce an acute phase response nor was the concentration substantially altered during bacterial infection, however both lectins were localized by immunohistochemistry in intimate association with microbes *in vivo*. The lectins were both found on mucosal surfaces and concentrated in leukocytes in blood and in inflammatory infiltrates. Antibody and primers are being devised to distinguish between RTLL-1 and -2 to examine differential expression.

Distinct Nonclassical MHC Class Ib Gene Lineages are Conserved in Divergent Amphibian Species

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Nonclassical MHC class Ib (class Ib) are heterogeneous genes encoding molecules structurally similar to classical MHC class Ia (class Ia) but with a more limited tissue distribution and polymorphism. In mammals, class Ibs have diverse, and often not well characterized, functions in CD8 T cell differentiation and regulation. Class Ib genes have been identified in all taxa of jawed vertebrates, but because of their rapid rate of evolution, their phylogeny is not well understood. The amphibian subfamily *Xenopodinae*, including the species *Silurana tropicalis* and *Xenopus laevis*, which diverged from a common ancestor ~ 65 million years ago, provides an alternative non-mammalian model to investigate the evolution and immune functions of class Ib genes over a period of evolutionary time comparable to the divergence between primates and rodents. We took advantage of the fully sequenced genome of *S. tropicalis* to identify, characterize and compare class Ib genes between this species and *X. laevis*, one of the best characterized non-mammalian animal models to study immunity. Genomic, molecular and phylogenetic studies reveal an unexpected degree of conservation of class Ib gene lineages in *Xenopodinae*. One unique lineage is represented by the divergent *X. laevis XNC10* gene and its unequivocal *S. tropicalis* ortholog, *SNC10*. Both *SNC10* and *XNC10* are expressed in larvae of their respective species by radiosensitive thymocytes from the onset of thymic organogenesis. Overall, this study highlights the unusual evolutionary conservation of class Ib gene lineages in *Xenopodinae*, and their possible involvement in the early development of CD8 T cells.

Research Support: F31-AI68610 (AG); R24-AI0598030 (JS); R03-HD061671(JR) from NIH

Characterization of innate immunoregulatory receptors in the channel catfish (*Ictalurus punctatus)*

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The overall objective of our research is to better understand the cellular and molecular aspects of innate immune responses by functionally characterizing a recently identified family of immune receptors in an alternative and unique animal model system, the channel catfish (*Ictalurus punctatus*). Termed leukocyte immune-type receptors (IpLITRs) these cellular proteins represent a highly diverse multigene family that are related to human innate immunoregulatory receptors vital for coordinating and controlling cellular immune responses. Putative stimulatory and inhibitory IpLITR-types are co-expressed by different catfish immune cells (e.g. NK cells, T cells, B cells, and macrophages) and like some mammalian IgSF receptors, select IpLITR-types are predicted to bind major histocompatibility class I proteins. Presently, no information is available regarding IpLITR signaling potential and their ligands remain to be identified. In this presentation, I will describe the features of the polymorphic and polygenic IpLITR family and provide data suggesting that these cellular proteins use conserved inhibitory and stimulatory signaling pathways to regulate immune cell effector functions. For example transfection of murine natural killer cells (LAK) with chimeric constructs encoding the ITIM-domain containing cytoplasmic tails of putative inhibitory IpLITR-types caused a significant reduction in LAK-mediated cytotoxicity towards specific target cells. In addition, stable transfection of rat mast cells (RBL-2H3) with stimulatory IpLITR-types revealed that these proteins use cellular activation pathways leading towards degranulation and phagocytosis. Combined with our previous biochemical studies indicating that distinct IpLITRs either recruit phosphatases or partner with ITAM-containing adaptors to inhibit or augment immune cell responses, respectively. This data represents an important first look into how IpLITRs regulate various immune cell effector functions and suggests that certain inhibitory signaling pathways are conserved among vertebrates.

Investigating the roles of hsp72 and hsc73 in MHC class I-mediated immune surveillance in the frog *Xenopus laevis*

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The heat shock proteins (hsps) gp96 and hsp70 mediate potent antigen-dependent anti-tumor responses in both mammals and *Xenopus*. We have developed an adoptive cell transfer assay using peritoneal leukocytes as antigen presenting cells (APCs), and shown that hsp72, but not hsc73, is as potent as gp96 to prime T cell responses *in vivo* against the *Xenopus* thymic tumor 15/0 that is tumorigenic when transplanted in MHC-compatible *Xenopus* clones. The 15/0 tumor expresses several nonclassical MHC class Ib (class Ib) genes and β2-m, however it does not express classical MHC class Ia (class Ia). Despite the lack of class Ia expression by 15/0, *Xenopus* adults generate potent cytotoxic unconventional CD8 T cells (CCU-CTLs) that specifically recognize and kill the 15/0 tumor, and that interact with class Ib molecules. We postulate that hsp72, but not hsc73, can prime class Ib-mediated anti-tumor CCU-CTL responses by interacting with APCs. To reveal the roles of class Ia and class Ib in this process we silenced surface expression of both molecules on APCs and assessed the effects in priming hsp72- or hsc73-mediated tumor immunity by adoptive transfer. To further elucidate the involvement of class Ia and class Ib in hsp70-mediated T cell function *in vivo,* we developed reliable transgenic techniques for our *Xenopus* clones and obtained F0 animals with silenced β2-m expression *in vivo*. This study will allow us to decipher the intricate relationship between hsps, class Ia and class Ib molecules in immune surveillance.

Research Support: *NIH T32-AI 07285 (H.N.), 1R03-HD061671-01, R24-AI-059830-06*

A simple model for systems-level innate immunity

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Many important hematopoietic and immune regulators are shared between vertebrate blood cells and sea urchin immunocytes. Expression of these factors suggests that although the protective strategies of these deuterostome groups differ markedly, they are integrated within a conserved regulatory and developmental framework. The sea urchin embryo and larva provide a morphologically simple experimental system for characterizing gene regulatory networks and we have initiated investigations of the interactions between immunocytes and other cell types using the larva as a simple infection model. The feeding larva has several cell types that carry out immune functions and we have characterized these cells in terms of gene expression and migratory behaviour. We have developed a gut-associated bacterial infection model to study feeding immunity. With this simple system we are characterizing the organism-wide response to infection. We observe a response in several cellular compartments distributed throughout the larva. This approach will provide a causal framework to characterize organism-level control of simple immune reactions to determine exactly what aspects of these systems are conserved among deuterostomes and how these conserved regulatory programs interface with divergent recognition and effector mechanisms. On a basic level this model will allow us to investigate fundamental interactions between immunocytes and the gut epithelium. From an evolutionary perspective regulatory mechanisms that operate to select and expand immune specificities may be shared between the complex innate immune system in the sea urchin and the intricate adaptive systems of vertebrates.

Thursday May 27th, 3.30-5.10 Evolution and Development of Immunity

Phylogeny and ontogeny of CD4, CD8α and CD8β T cell co-receptor homologs in two amphibian species: *Silurana tropicalis* and *Xenopus laevis*

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In many jawed vertebrates CD4, CD4-like and CD8 genes have been identified, but the phylogenetic and functional conservation of these molecules is not fully understood. We have characterized CD4, CD8α and CD8β gene homologs in two species of the subfamily *Xenopodinae*, *S. tropicalis,* whose full genome is sequenced, and *X. laevis*. Multiple alignments of amino acid sequences, in these two species, as in other nonmammalian species, reveal a poor conservation of the residues of CD4 and CD8α that, in mammals, bind to MHC class II and class I molecules, respectively. This is consistent with a species-specific coevolution of these genes. Phylogenetic and syntenic analysis indicates that all *Xenopodinae* three co-receptor genes are more related to other tetrapods than to bony fish. Furthermore, developmental and cell-specific expression patterns of these genes in *X. laevis* are very similar to that of mammals. *X. laevis* CD4 is mainly expressed by peripheral non-CD8 T cells and detected in the thymus at the onset of organogenesis, 5 days post-fertilization (dpf). The CD8β gene is specifically expressed by adult CD8 positive T cells and thymocytes, and is first detected in the thymus and intestine at 6 dpf. TCRα and TCRγ transcripts are also detected at 6 dpf, before TCRβ (9 dpf). TCRγ transcripts cloned from 7dpf are fully rearranged and in frame. This suggests that T cell subsets, possibly γ/δ T cells or even α/βCD8 T cells, reside in the intestine before the onset of thymocyte differentiation, and may contribute to mucosal immunity early in development.

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The cellular context of AID expressing cells in fish lymphoid tissue.

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It has long been held that the cold- blooded vertebrates lack mammalian-like germinal centers, though they do have affinity maturation and the immunoglobulin mutator activation-induced cytidine deaminase or AID. Using AID as a marker of sites of somatic hypermutation, we have identified discrete cell clusters of up to several thousand cells, in the spleen and kidney of channel catfish (Ictalurus punctatus), which may be primordial germinal centers. In situ hybridization revealed that AID expressing cells are interspersed or surrounded by a population of pigmented CSF1-R expressing cells called melano-macrophages. Significantly, melano-macrophages have been previously noted for their ability to retain soluble antigen on or near their surface for several weeks following vaccination. Laser capture micro-dissection and RT-PCR were used to establish that these cell clusters also contained cells expressing Ig heavy chain transcripts as well as transcripts of TcRb and the putative CD4 homologue of fish. These observations, coupled with past work showing that mutations develop in B-cell lineages in fishes, allow us to develop a model for how affinity maturation evolved in early gnathostome vertebrates.

Gata and bHLH factors form a conserved regulatory circuit for deuterostome immunocyte development

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Two major cell types carry out immunity in the purple sea urchin larva: pigment cells, which migrate to sites of infection and injury, and sub-sets of the blastocoelar cells, which phagocytose foreign particles, and express a suite of immune receptors and effectors. Specification of immunocytes occurs in a ring of cells localized in the vegetal plate of the mesenchyme blastula. We have identified homologues of key vertebrate hematopoietic transcription factors, with expression profiles consistent with a role in sub-specification of these cells. These include Sp-Gatac, a zinc-finger transcription factor homologous to vertebrate Gata-1,-2 and -3 and a homolog of the Scl, Tal2 and Lyl1 members of the bHLH family, Sp-Scl, which play distinct roles in immunocyte precursor sub-specification in the sea urchin embryo. Sp-Gatac perturbation has a profound effect on blastocoelar cell ingression, while Sp-Scl knock-down results in a signal dependant, non-cell autonomous pigment cell defect. Other components of the vertebrate hematopoietic gene regulatory network are also expressed in these cells, including Sp-E2A, homologous to vertebrate hematopoietic E-proteins E2A, HEB and ITF-2, an Id-1/2/3/4 homologue, Sp-Id, and the cofactor Sp-Lmo2. This suggests the existence of a conserved gene regulatory circuit for early immune cell development, involving GATA and bHLH transcription factors that are active even in the very simple developmental context of the sea urchin embryo.

Evolution of duplicated *IgH* loci in Atlantic salmon (*Salmo salar*)

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The Atlantic salmon (*Salmo salar*) immunoglobulin heavy chain (*IgH*) locus possesses two parallel *IgH* isoloci (*IGH-A* and *IGH-B*), that are related to the genomic duplication event in the family Salmonidae approximately 25 - 100 mya. In this study, we defined the structure of these loci in Atlantic salmon, and sequenced 24 bacterial artificial chromosome (BAC) clones that were assembled into the *IGH-A* (1.1MB) and *IGH-B* (0.9) Mb loci. In addition, over 7,000 cDNA clones from the *IgH* variable (Vh) region have been sequenced and analyzed. The present study shows that the genomic organization of the duplicated *IgH* loci in Atlantic salmon differs from that in other teleosts and other vertebrates. The loci possess multiple Cτ genes upstream of the Cμ region, with three of the Cτ genes being functional. Moreover, the duplicated loci possess over 300 Vh segments which could be classified into 18 families. This is the largest number of Vh families currently defined in any vertebrate. There were significant structural differences between the two loci, indicating that both *IGH-A* and *-B* loci have evolved independently in the short time after the recent genome duplication approximately 25 - 100 mya. Our results indicate that the duplication of the *IgH* loci in Atlantic salmon significantly contributes to the increased diversity of the antibody repertoire, as compared with the single *IgH* locus in other vertebrates.

Characterization of lipid rafts in fish immune cells

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In mammalian immune cells lipid rafts play a significant role in the initiation of signal transduction mechanisms through several immune receptors. Mammalian lipid rafts are characterized by the presence of different markers. Lipidic markers include cholesterol and gangliosides such as GM1, which are believed to be lipid raft structural components. Protein markers include cavelolin, clathrin, and flotillin. These proteins are required for immune receptor signaling, and endocytosis. We used primary kidney-derived goldfish macrophages, a catfish B-cell line and catfish peripheral blood lymphocytes, to biochemically characterize possible lipid rafts markers in fish. The level of plasma membrane cholesterol and GM1 was analyzed by flow cytometry using fluorescent probes. Catfish B lymphocytes show levels cholesterol similar to those of mammalian lymphocytes, but displayed drastically reduced levels of GM1. Progenitor cells, monocytes, and macrophages derived from primary kidney leukocyte cultures of golfish had significantly lower levels of cholesterol compared to mammalian macrophages. During the initial lag phase (non-proliferative) of in vitro development goldfish macrophages had GM1 levels comparable to those of mammalian macrophages. Interestingly, GM1 level decreased progressively, as the cells entered the proliferative phase of in vitro development, acquiring a more mature phenotype. Western blotting experiments showed that antibodies that recognized mammalian cavelolin, clathrin, and flotillin also recognized catfish lymphocyte and goldfish macrophage proteins. Based on protein sequence alignments of mammalian and fish cavelolin, clathrin, and flotillin, and our Western blot results, it appears that these proteins may have conserved functions in mammalian and fish lipid rafts, despite the lipid biochemistry differences. Our findings suggest that the biochemistry of fish lipid rafts is distinct from mammalian lipid rafts, and raise the possibility that lipid raft expression and assembly is regulated during development of fish macrophages in vitro. To our knowledge, this is the first attempt to biochemically characterize lipid rafts in immune cells of lower vertebrates. [Support: NSERC, Canada]

Friday May 28th, 10.30-12.00 Environment and Husbandry

Comparing the Immune Response of the Brown Bullhead Catfish (*Ictularus nebulosus)* from Clean and Contaminated Sites along the Detroit River

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Some fish populations are able to adapt and thrive in contaminated habitats. Survival of populations depends on the ability of the organism to elicit resistance, either due to genetic adaptation or physiological acclimations. Brown Bullhead catfish (*Ictularus nebulosus*) are able to survive in very contaminated areas and due to their benthic and philopatric characteristics make them a model organism to study chronic exposure. This research attempts to assess the immune function of the brown bullhead catfish at four pre-determined sites along the Detroit River, which are chronically exposed to high or low concentrations of environmental toxicants. Clean and contaminated sediment used for contaminant exposure was collected by ponar at designated sites of the river. The Bullheads were vaccinated with heat killed *V. anguillarum* in order to induce an immune response and divided evenly into corresponding contaminant exposure tanks. Respiratory Burst assays to assess innate oxygen radical production, enzyme linked immunosorbant assay (ELISA) to assess antibody production, and real time PCR to assess immune gene expression have been undertaken. To date, respiratory burst data 24 hours post sediment exposure and vaccination has shown an inhibition of neutrophil oxidative activity in adult cleared bullheads collected from clean sites placed on contaminated sediment compared to those placed on clean sediment, while no inhibition was identified in contaminated fish placed on either sediment. Results suggest a genetic or physiological change in the immune function which will be examined further in subsequent experiments.

Vaccination of Neonatal Calves: Obstacles and Opportunities

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Vaccination at a young age is desirable in many species as a means to induce early protection against infectious diseases. Unfortunately, limited immune function in neonates, combined with immunosuppressive effects of maternal antibodies (MatAb) make neonatal immunization difficult. Most studies of neonatal immune competence use human umbilical cord blood cells or neonatal mice as models of convenience. Cord blood cells reflect immune parameters during a narrow window, when concentrations of corticosteroids are particularly high, and are not representative of neonatal immune capacity. Immune competence of newborn mice is comparable to that of ***fetal*** humans. Newborn calves have a higher level of immune competence, and longitudinal studies of blood lymphocytes are practical, facilitated by the large blood volume.
Characteristics of blood IgM^+ B lymphocytes were assessed in 15 calves in a longitudinal study using flow cytometry, from the first week of life up to 6 months of age, to clarify obstacles to successful vaccination. Mean IgM^+ B cells increased from 1.3 x 10^8/L in week 0 to 2.7 x 10^9/L at 6 months. Intensity of expression of IgM was significantly higher in newborn calves, than in adults. Over 90% of circulating IgM^+ B cells expressed both CD21 (complement receptor 2, an activating receptor) and CD32 (FcγRII, an inhibitory receptor binding MatAb-antigen complexes) from the first week of life, suggesting the potential for both positive and negative regulation. Similar to humans (but in contrast to mice), B lymphocytes of neonatal calves express both the b1 and b2 sub-isoforms of CD32. Large animal models of neonatal immunology can yield information not accessible with more conventional approaches.

Breeding strategy and early rearing environment affect the immune response in Chinook salmon (*0ncorhynchus tshawytscha*).

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Salmon aquaculture practices are fundamentally based on artificial mating, which in time can lead farmed stocks to diverge genetically and phenotypically from wild fish. In this context, the fish immune system can also be affected and is of extreme importance for commercial fish production. The work presented here aims to increase our general knowledge of the biology of this species by taking an immunological approach. A disease challenge using *Vibrio anguillarum* was performed on four groups of fish which combined two different breeding strategies (artificial vs. semi-natural) with two rearing environments (indoor artificial tanks vs. outdoor gravelled channels). Results including mortality rates and MH class II β1 genotyping indicate that fish immune responses are affected by breeding strategies and early rearing environments. In particular, differences in mortalities are accentuated in artificially-spawned fish, while semi-naturally-spawned fish does not seems to be severely affected by different rearing environments. Moreover, there are no clear associations of survival or mortality to particular alleles or genotypes of the MH class II antigen-presenting molecules. Gene expression analysis employing a 700-gene chip cDNA microarray on samples from control and infected fish is currently being undertaken which will hopefully help to assess the fish immune response at the transcriptome level. The use of semi-natural spawning procedures may improve the quality of the farmed fish by providing them with a better chance to face diseases in spite of environmental changes.

Interaction between pathogen rickettsia-like organism and its host, oyster *Crassostrea ariakensis*

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A series of innate immune molecules were found that they might be involved in immune responses against rickettsia-like organism （RLO）. These molecules included CREB, CasTRAIL, TNF- alpha, Myd88, Mitogen-activated protein kinase (p38, JNK, ERK), NF- κ B, CaTLL(TLD-like metalloproteinases). Here we can take our some examples to describe the important progress in this field:It has been proved that the evidence and probably mechanism of interaction between RLO *omp*R and host defense system as follows: (1) It was suggested that JNK and p38 mediated signaling pathways might involve in the immune responses induced by a RLO outer membrane protein (*omp*R); (2) p38 and Myd88-mediated signaling pathways could lead to activation of NF- κB transcription factors and the expression of downstream gene TNF- alpha according to the DNA binding activity of NF- κB in hemocytes apparently induced by *omp*R and inhibited by inhibitor of the p38 pathway. According to the results of the phosphorylation state of MAP kinases, it revealed that CasTRAIL induced a rapid increase in the phospho-ERK and phospho-p38 levels, which indicated that the MAPK pathway was involved in CasTRAIL-mediated signaling. In addition, CasTRAIL also showed an ability of anti-RLO infection which might be through the p38-MAPK activation pathway. About CREB responses to the challenge of rickettsia-like organism (RLO), the results showed that the expression level of Ca-CREB in hemocytes was not modified remarkably by RLO. However, the expression level of TNF-a was increased obviously, suggesting that the immune response had been induced by RLO. In addition, DNA-binding activity and phosphorylation of Ca-CREB were obviously induced by RLO. Therefore, according to above results, it has been proved that the regulation of Ca-CREB did not occur at the transcriptional level or the expression level could not be modified by RLO, in other words, Ca-CREB was regulated at the protein level, instead of the mRNA level, after RLO stimulation. In summary, from all described above support the hypothesis that RLO pathogen is through activation of p38-MAPK and NF-κB transcription factor to trigger a role against infection and it is tempting to infer that a MAPK/CREB pathway may exist in oyster and contribute to the immune response for RLO.

Friday May 28th, 1.00-3.00 Macrophage Function and Immune Gene Expression

Macrophage activation differentially modulates binding, internalization and downstream killing responses during phagocytosis

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Induction of phagocytic responses provides a potent innate strategy for the destruction of invading pathogens. Phagocytes have the capacity to differentially regulate binding and internalization processes through changes in their receptor profile and modulation of downstream events. This is necessary for the intricate control of phagocytic antimicrobial responses. Several methods are available for evaluation of phagocytosis. Unfortunately, none allow for accurate quantitation of both binding and internalization events. To overcome these limitations, we have developed a novel phagocytosis assay based on a multi-spectral imaging flow cytometry platform. We have also devised a novel approach for examination of phagolysosome fusion and provide the first quantitative assessment of phagolysosome fusion in any cellular system. Importantly, our approaches are amenable to a broad range of comparative model systems. In this presentation I will show the application of these novel assays for the characterization of phagocytosis in a teleost fish primary kidney macrophage (PKM) model. While it has been previously reported that mixed populations of these cultures are phagocytic, it has remained unclear if sub-populations within these cultures contribute differentially to this activity. The temporal activation of specific phagocytic antimicrobial responses at distinct stages of teleost macrophage differentiation suggests specialization within the macrophage compartment early in evolution, geared to meet specific host immunity requirements within specialized niches.

Key words: innate immunity, macrophage, phagocytosis, comparative model systems

This study was supported by the Natural Sciences and Engineering Research Council of Canada, and the Alberta Agricultural Research Institute.

Assessment of macrophage antimicrobial responses induced by type II interferons of the goldfish (*Carassius auratus* L.)

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Unlike mammals, bony fish have two type II interferons, IFNγ and IFNγrel, whose pro-inflammatory functions have not been fully characterized. To examine the antimicrobial responses induced by type II interferons of bony fish, we measured gene expression and functional responses of goldfish mononuclear phagocytes after stimulation with recombinant goldfish (rg) IFNγ and rgIFNγrel. Our findings indicate that rgIFNγrel possesses novel as well as functionally redundant roles shared with rgIFNγ. While rgIFNγ primed goldfish monocytes for enhanced reactive oxygen intermediate (ROI) production, rgIFNγrel down-regulated the ROI priming effects mediated by rgIFNγ and rgTNFα2. This inhibitory effect was abolished after addition of anti-rgIFNγrel affinity-purified IgG. The rgIFNγ induced relatively modest phagocytic and nitric oxide responses in goldfish monocytes and macrophages, respectively. In contrast, rgIFNγrel induced highly significant and robust phagocytosis, iNOS gene expression and nitric oxide production compared to rgIFNγ. The expression analysis of select immune genes of monocytes and macrophages revealed distinct expression profiles in cells treated with rgIFNγrel and those treated rgIFNγ. These differences included significantly higher induction of TNFa2 and CXCL8 expression in activated monocytes and macrophages. Our findings suggest the presence of functional segregation of the induction of macrophage antimicrobial functions by type II interferons of bony fish.[Funded by NSERC, Canada]

Critical role of macrophages in amphibian immune surveillance

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Although the involvement of B and T cells in immune responses has been extensively studied in *Xenopus*, little is known about the role of innate effectors such as macrophages in pro-inflammatory responses and antigen (Ag) presentation. We have investigated whether peritoneal macrophages (pMs) can function as innate cell effectors as well as antigen presenting cells (APCs). To address APC function, we have developed an *in vivo* priming technique by adoptive transfer, where pMs are pulsed with minor histocompatibility (H) Ags complexed to the stress protein gp96. Our data demonstrate that *Xenopus* pMs are efficient APCs able to cross-present minor H-Ags and promote T cell-dependent accelerated skin graft rejection. To assess innate immune function, we characterized the response of pMs during infection with the ranavirus FV3. Our results show that the relative number of activated pMs rapidly increases as early a 1 day post-infection, concomitantly with up-regulation of the pro-inflammatory genes Arginase 1, IL-1b and TNFąIn addition, while pMs are susceptible to FV3 infection as evidenced by apoptotic cells, active FV3 transcription and the detection of viral particles by electron microscopy, the infection is weaker (fewer infectious particles) and more transitory than the kidney, the main site of infection. Moreover, viral DNA remains detectable in pMs for at least 3 weeks post-infection, past the point of viral clearance observed in the kidneys. This suggests that although pMs are actively involved in anti-FV3 immune responses, some of these cells can be permissive and harbor quiescent, asymptomatic FV3.

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Development of an Atlantic cod (*Gadus morhua*) oligonucleotide microarray and its validation using a study of cod spleen global gene expression responses to stimulation with formalin-killed atypical *Aeromonas salmonicida*

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A goal of the Atlantic Cod Genomics and Broodstock Development Project (CGP) is to develop fundamental genomic resources and tools to use in research on cod responses to environmental stress and viral, bacterial and fungal pathogens. The CGP has generated ~160,000 Atlantic cod ESTs (assembled into ~50,000 unique sequences) from 23 normalized and 19 SSH libraries, representing 12 tissues and 4 developmental stages. The SSH libraries were generated using samples from fish exposed to stress or immune stimuli such as nodavirus and formalin-killed atypical *Aeromonas salmonicida*. From this EST collection, representative sequences were selected for the construction of a 50-mer oligonucleotide microarray containing 20,000 unique elements. Selection of sequences was performed to maximize the number of sequences with functional annotation or homology to sequences in the GenBank database. Other selection criteria included a high degree of representation in the EST database and known directionality. Sequences from the SSH libraries without significant homology to GenBank entries were included to represent unknown genes with putative functions in stress or immune responses. Three small pilot experiments (4 slides each) using tissues from cod injected with viral mimic poly (I:C) or cod with asymptomatic nodavirus infection confirmed that there were no systematical errors in array layout and probe design and that different chemistries can be used with the array. A larger, 40-slide experiment investigated the transcriptome responses of cod spleen to stimulation with formalin-killed atypical *Aeromonas salmonicida.* Among the genes showing the strongest upregulation by formalin-killed atypical *Aeromonas salmonicida* injection were CC chemokines and antimicrobial peptides. Differentially expressed genes identified in this experiment were compared to genes identified in an SSH study that used the same cod spleen samples. This experiment is the definitive validation of the CGP 20K microarray platform, which will provide a valuable tool for future research on cod biology.

Upregulation of immune genes in duck lung and intestine during high and low pathogenic avian influenza infection

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Ducks are the natural reservoir of influenza A virus and survive infection by most strains. Humans and chickens are more susceptible to disease. It is important to understand what constitutes a successful antiviral response in influenza's environmental host. Since influenza is an acute infection the innate immune system is likely critical in viral clearance and host survival. To elucidate the host-pathogen interactions that occur during influenza A infection we are examining host genes upregulated early in an immune response. Suppressive subtractive hybridization (SSH) was used to determine which genes are upregulated one day post infection with high and low pathogenic avian influenza in ducks. RNA from lung or intestine tissue of ducks infected with either high (H5N1/VN1203/04) or low (H5N2/BC2005/500) pathogenic strains of influenza A virus was compared to that of mock-infected animals by SSH. Both VN1203 and BC500-infected lung and intestine tissues showed increased expression of a wide variety of immune genes during influenza infection, including MHC class I, interferon induced protein with tricopeptide repeats 5 (IFIT5), 2'-5' oligoadenylate synthase (OAS), interferon induced transmembrane proteins (IFITMs), activation-induced C-type lectins (AICLs), heat shock proteins, as well as several housekeeping genes. The differential expression of these genes during an immune response to influenza was confirmed by reverse transcription PCR (RT-PCR) and dioxygenin-dUTP (DIG) dot blotting. Real time qPCR analysis of OAS and IFIT5 showed greater than 1000-fold upregulation of these interferon stimulated genes in duck lung following VN1203 infection, and much less upregulation by BC500 infection. Our results suggest that ducks mount a more robust response to potentially lethal influenza viruses. Identifying genes involved in a successful antiviral response in the natural host may reveal new targets for therapeutic intervention in human and veterinary disease.

Functional Characterization of the Rainbow Trout (*Oncorhynchus mykiss*) Chemokine, CK-2

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The rainbow trout chemokine CK-2 is the only known CC chemokine in possession of a mucin stalk. CK-2.1 is a related rainbow trout CC chemokine that includes a longer mucin stalk and was originally thought to be a separate gene. Genotyping of outbred individuals showed that CK-2.1 is probably an allele of CK-2. A previous study showed that stimulation by PHA causes a decrease in CK-2 transcript levels in rainbow trout head kidney and peripheral blood leukocytes (PBLs) as well as the rainbow trout macrophage-like cell line RTS-11. CK-2 protein was found expressed in RTS-11 but not in the spleen tissues of stimulated fish. A chemotaxis assay was performed to determine the activity of recombinant CK-2 and CK-2.1. It was observed that CK-2 induces the migration of rainbow trout PBLs as well as RTS-11 cells. Treatment of RTS-11 cells with recombinant CK-2 results in changes in expression profiles of various immune response genes. This study shows that CK-2 is a functional chemokine that has a role in rainbow trout immune response involving, but not limited to, macrophages.

Poster Session Thursday May 27th 7.00-10.00pm

1. Identification and functional characterization of a novel Rbx1 in an invertebrate *Haliotis diversicolor supertexta*

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Rbx1 (RING box1) is an evolutionarily conserved RING-H2 finger protein and belongs to the RING-finger family of Ubiquitin ligase E3, which determines the substrate specificity of ubiquitination and regulates a variety of biological processes. We report here the identification and functional characterization of an Rbx1 homologue in abalone, which we named ab-Rbx1. Ab-Rbx1 contains conserved cysteine/histidine residues which are the characteristics of Rbx proteins. Phylogenetic tree analysis further demonstrated ab-Rbx1 belongs to the Rbx1 family other than Rbx2 family. Real-time PCR analysis revealed that ab-Rbx1 was ubiquitously expressed in all examined tissues of abalone and the expression level of ab-Rbx1 was significantly induced by mitogenic situation. Immunohistochemical and immunofluorescent staining showed that the ab-Rbx1 was expressed predominantly in epithelial cells and localized both in the cytoplasmic and nuclear compartment. Ubiquitination assay demonstrated that ab-Rbx1 had ubiquitin ligase activity and could be auto-ubiquitinated by itself. These results suggest that ab-Rbx1 is an Rbx1 homologue and may be indirectly involved in the immune response of abalone through ubiquitination.

2. Investigation of MH Class I Alleles in *Melanogrammus aeglefinus*

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Melanogrammus aeglefinus, commonly known as haddock, is a commercially important marine fish species closely related to cod. Preliminary investigations into the immune function of this species has revealed several unique and interesting features, including an unusually high number of expressed alleles of Major Histocompatibility (MH) Class I genes. The goal of this project was to examine the sequences of alleles, including the untranslated regions, for potential regulatory mechanisms which may limit the number of alleles expressed to the point of functional molecules. There are two key putative mechanisms. The first is the inversion of the open reading frame within the transcript. The second is the linking of the MH Class I transcript with the transcript of another gene. Using a cDNA library created from the head kidney, spleen, liver and muscle from one individual MH Class I alleles were isolated and sequenced. A total of 22 unique alleles have been identified from the library. Of these alleles, one has an inverted open reading frame, 11 are completely inverted including the UTRs, and four are joined with sequences of other proteins.

3. Diagnostic fish health; new agents new challenges new models

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Fish disease diagnosis can present a challenge due to lack of cell lines and other tools. With a range of novel fish species in culture and the diversity of teleosts, new diseases and agents present themselves with surprising frequency. The fish pathology laboratory receives a restricted caseload yet over the past several years several new diseases and or disease presentations have been discovered. These include viral hemorrhagic septicemia virus in the Great Lakes, koi herpes virus in Canada, white sucker virus – an uncharacterized yet readily culturable virus associated with mortality in brown bullhead and suckers, and suspected viruses of cultured kingfish and hapuku from New Zealand. Necrotizing dermatitis or 'redtail' of seahorses, chlamydia-like agent of Lake and brook trout and *Sanguinicola* sp. endocarditis of walleye and sauger are other infectious diseases. Non-infectious entities include gastric dilation and air sacculitis of sturgeon fed rainbow trout chow, 'no mucus skin disease' of walleye, a nutritional myopathy of seahorses and a neoplasia-associated amyloidosis of redtail sharks. Several of these are now subject of ongoing research.

4. The Effect of Brominated Bisphenols on the Rainbow Trout Macrophage Cell Line RTS11

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Although the plastic congener bisphenol A (BPA) has received much scientific and public attention, a related group of compounds also are of interest for their potential impact on environmental and human health. These are the brominated bisphenols, which differ from bisphenol A (BPA) only by placement of bromine constituents on its aromatic rings. Mono-, di-, tri- and tetra- substituted forms of bisphenol A are possible: monobromobisphenol A (MBBPA), dibromobisphenol A (diBBPA), tribromobisphenol A (triBBPA), and tetrabromobisphenol A (TBBPA), respectively. Of these, TBBPA is the most common compound, used as a flame retardant in a long list of products including plastic computer components. Breakdown of TBBPA can yield each of the less brominated forms (triBBPA, diBBPA, MBBPA and BPA) and these have been detected in samples from aquatic environments. As TBBPA has been shown to be toxic in some studies, alternatives to TBBPA have been sought and one of these is tetrabromobisphenol A bis (2,3-dibromopropylether) (TBBPA-DBPE). Little or nothing is known about the toxicity of this compound or of the less brominated forms. Therefore a study of the effect of these compounds on rainbow trout cells was undertaken. Several cell lines have been examined but the emphasis has been on the macrophage-like cell line, RTS11, because others have noted effects on the macrophages of mammals for at least BPA. Cytotoxicity was evaluated for RTS11 with Trypan blue and for the adherent cell lines with three fluorescent indicator dyes. With the exception of TBBPA-DBPE, which was not cytotoxic, all compounds were cytotoxic above approximately 2 μg/ml in all the cell lines and with all measures of cell viability. For RTS11, the effects of these compounds on phagocytosis, cell attachment, and proliferation continue to be studied at concentrations below 2 μg/ml, with no effect seen to date on proliferation.

5. Genetic markers of the immune response of Atlantic salmon (*Salmo salar*) to Infectious Salmon Anemia Virus (ISAV)

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Infectious diseases among fish present an important economic burden for the aquaculture and fisheries industries around the world. For example, the infectious salmon anemia virus (ISAV) infects 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 the losses and the number of outbreaks, the risk of new virulent strains emerging is a looming threat to the viability and sustainability of this industry. A greater understanding of the host-pathogen interactions at the molecular level during the course of an infection thus remains of strategic importance for the development of molecular tools and efficient vaccines capable of minimizing losses in the eventual case of a new outbreak. Using a 32 k cDNA microarray platform (cGRASP), we have identified various signaling pathways and immune regulated genes, which are activated or repressed in Atlantic salmon head-kidney during the course of an ISAV infection. Gene expressions were measured at five different time-points: 6h, 24h, 3d, 7d and 16d post injection. The earliest time points showed only a few differentially expressed genes in ISAV injected fish, relative to sham injected controls, although as time progressed, many additional genes involved in key defense pathways were up-regulated including MHC type I, beta-2 microglobulin, TRIM 25 and CC chemokine 19. During the latest stage of the infection process, many genes related to oxygen transportation were under-expressed, which correlates well with the observed anemia that occurs prior to death in Atlantic salmon infected with virulent strains of ISAV.

6. Interactions between fish macrophages and viral hemorrhagic septicemia virus, Type IV strains

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Viral hemorrhagic septicemia virus (VHSV) is an enveloped, single stranded, negative-strand RNA virus in the *Rhabdoviridae* family. VHSV belongs to the *novirhabdovirus* genus and is classified into four genotypes: I, II, III and IV. Genotype I is most virulent to rainbow trout, whereas types IV seem much less harmful to this species. Yet type IVa is virulent to marine fish, like Pacific herring; type IVb, to a variety of fresh water fish, such as walleye. A major component of innate immunity against viral infections is the macrophage. The most famous rhabdovirus, rabies, has a complex relationship with macrophages, with most strains being unable to replicate in macrophages. The relationship between VHSV strains and macrophages is just beginning to be understood and is the goal of this study. Type I underwent an aborted infection in the rainbow trout macrophage cell line, RTS11. Cells expressed transcripts for viral N and G genes as determined by RT-PCR and N protein as determined by western blotting. In RTS11, Type I induced transcripts for Mx1, Mx2, Mx3, IFN-1 and IFN-2 but not transcripts for IL-1β, IL-8, TNF-α1, IFN-γ, and iNOS. RTS11 cultures did not undergo cytopathic effect (CPE) and failed to produce virus. By contrast, types IVa and IVb failed to undergo even an aborted infection in RTS11 as viral transcripts could not be detected in virally exposed cultures. This result suggests that Type IV strains of VHSV either cannot enter VHSV, due to incompatible virus/cell receptors, or enter but do not initiate transcription. Currently the ability of type IV VHSV to induce gene expression in RTS11 is being investigated. Transcripts for three groups of genes are being examined: genes for proinflammatory cytokines, such as IL-8; antiviral mechanisms, such as Mxs; and major histocompatibiliy complex polypeptides, such as β2 microglobulin.

7. Evaluation of the roles of putative viral genes in virulence and immunity of Frog virus 3 in *Xenopus laevis*

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Global declines in amphibian population are perhaps one of the most pressing and enigmatic environmental problems. Understanding the causes of amphibian decline is of public interest, and may reveal practical ways to protect endangered amphibian species from infectious agents. Ranaviruses (RV), family *Iridoviridae*, have become increasingly prevalent pathogens of amphibians around the world, and are possibly involved in the worldwide decline of amphibian populations. Nonetheless, RVs have not yet been studied as intensively as other large DNA viruses. We have established the frog *X. laevis* as an important experimental model to study viral pathogenicity and host defenses against RVs such as Frog virus 3 (FV3), and evaluate the contribution of immunocompromised animals in the dissemination of the diseases. To investigate the possible role of viral genes in virulence and immune evasion, we are currently developing a method to systematically knockout (KO) putative virulence genes by site-specific integration of a selectable fluorescent marker into the FV3 genome. Susceptible *X. laevis* larvae will provide an ideal model to evaluate the impact of KOs on *in vivo* virus load, host mortality and the induction of pro-inflammatory genes. Normal and immunocompromised adult frogs will be used to investigate pathogenicity and ability of KOs to evade adaptive immunity. These studies will help elucidate the interplay between virulence genes and host immune defenses and may reveal original mechanisms of host-pathogen interaction that may be relevant not only to amphibians and other lower vertebrates, but also to humans.
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8. Examining the immune response to viral hemorrhagic septicemia virus (VHSV) in *Sander vitreus*

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Viral hemorrhagic septicemia (VHS) is a disease currently affecting both marine and freshwater fish worldwide. Caused by the viral hemorrhagic septicemia virus (VHSV), this disease is of great economic importance in Canada, where fisheries (commercial and recreational) bring in about $11.4 billion annually. Over 25 species in all 5 Great Lakes have been affected by VHS. The purpose of this research is to provide information needed to manage *S.vitreus* (walleye) stocks with the continued presence of VHSV in the Great Lakes by examining the immune reaction of the fish to VHSV infection.DNA sequencing of major histocompatibility genes will establish a genetic immunological basis for resistance and susceptibility of different *S.vitreus* strains, while qPCR and western blotting will determine what gene expression patterns constitute protective (P) and non-protective (NP) responses in these fish. Effects of temperature and stress will be monitored by qPCR and western blotting in P and NP responses during infection trials to examine changes in gene expression. A previously reported neutralizing antibody response to VHSV vaccination will be examined during infection trials to assess occurrence of this response in naturally infected *S.vitreus* using qPCR and western blotting. Primers for PCR reactions that will assess levels of expression of immune genes have been designed and tested and recombinant protein for the development of antibodies to be used in western blot assays has been initiated. Results of this research will provide the base knowledge for effective stocking strategies that produce resistant fish having increased survivability in the infected waters of the Great Lakes.

9. Susceptibility and immune responses of the *Xenopus laevis* larvae to an emerging ranavirus pathogen

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Emerging infectious diseases caused by ranaviruses (RVs) are increasingly prevalent and possibly involved in the worldwide decline of amphibian populations. We are using Frog Virus 3 (FV3) and the frog *Xenopus laevis* as a valuable experimental model system to investigate the interaction between RVs and the amphibian host immune system. Our previous studies have revealed significant differences in susceptibility and immune responses to FV3 between adults and larvae. Adult frogs develop a potent adaptive immune response and clear FV3 within 2-3 weeks. In contrast most tadpoles (90%) are unable to clear the virus and die within a few weeks after infection. We postulate, however, that FV3 can stimulate some immune responses in larvae. To assess this possibility, we determined the expression profile of genes relevant for innate and adaptive immune responses in various tissues of pre-metamorphic larvae (stage 56-57) at different times following FV3 infection by RT-PCR. Preliminary results indicate that expression of innate type genes such as TNFα, IL-1β and Arginase 1, as well as the adaptive type gene IFNγ, increases mainly in the liver at 6 days post-FV3 infection in larvae. This response is delayed in comparison to adults, where up-regulation of these genes is already detected 1 day post-infection. This suggests that larval susceptibility to FV3 is in part due to the delayed kinetics of the immune response. Furthermore, since viral transcription is mostly detected in the liver and not the kidneys as in adults, this organ may constitute both the major site of immune responses and the main target of FV3 infection.

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10. Characterizing the immune response to *Flavobacterium pyschrophilum,* and possible resistance/susceptibility conferring major histocompatibility alleles in *Oncorhynchus mykiss.*

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Coldwater disease is an increasingly prevalent issue and has a deleterious impact on farmed populations in Canadian aquaculture. Mortalities can approach approximately 80% in infected populations, causing significant monetary losses. However, the immune response to *Flavobacterium psychrophilum,* the causative agent of coldwater disease in rainbow trout, has yet to be fully elucidated. This project will assess that response using several measures. Firstly, Major Histocompatibility genes, genes that play an important role in initiating the immune response and specific alleles of these genes can be related to resistance or susceptibility to disease. Thus DNA of individual rainbow trout from six British Columbia strains will be extracted and sequenced to identify allelic differences between subpopulations. Secondly, the immune response to the bacteria will be characterized utilizing various immunological assays such as chemiluminescent detection of oxygen burst responses, qPCR for comparing immune system gene expression levels and ELISA for assessing antibody production. Thirdly, since there is anecdotal evidence that triploid fish are more susceptible to disease, the differences in immune response between triploid and diploid fish will be examined using the techniques mentioned above, as well as quantifying and comparing the mature immune cell populations. While these tests are currently underway the outcome of this project will be able to provide concrete results and aide in the production of new management tools for the aquaculture industry. This will lead to an overall decrease in the loss of farmed rainbow trout and a greater control over coldwater disease in the aquaculture industry.

11. *Sanguinicola occidentalis* In Walleye And Sauger From Lake Winnipeg, Canada.

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The Red River International Joint Commission undertook a three-year study of the impact of drainage from Devil's Lake to Lake Winnipeg, via the Cheyenne and Red Rivers. Ten species of fish were examined from Lake Winnipeg for bacteriology, virology and parasitology. The FPL undertook the light microscopy portion of the survey. Six hundred fish were examined in 2006-07 and 300 further fish (60 and 30, respectively of each species) in 2007-08. A particularly notable collection of lesions was found to affect walleye and to a lesser degree sauger. There was a moderate to severe proliferative endocarditis extending to include the heart valves, which was associated with few luminal trematodes. There was also a multifocal myocarditis and the inflammation was centered on trematode eggs. Eggs were also very commonly found lodged in pillar cell channels of branchial lamellae. Sixty-two and forty-two percent of the walleye and sauger, respectively, from a sample collected in summer had trematodes or typical lesions present. Twenty percent of both species were affected from a sample taken during spring. *Sanguinicola occidentalis* was subsequently identified in the hearts of walleye from Lake Winnipeg. *S. occidentalis* was not previously known to exist in Lake Winnipeg and the prevalence and severity of lesions suggest that its presence affects fish fitness.

12. A gene regulatory network model for gut-associated immunity

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The gut-associated immune system allows for rapid response to ingested pathogens while maintaining tolerance to symbiotic microbiota, and food and other non-self molecules. This immune circuitry must be tightly controlled as misregulation can lead to autoimmune effects or invasion by opportunistic microbes. Given the complexity of the gut structure and immune system in vertebrates, a simple invertebrate model is useful to characterize gut-associated immunity in the context of an intact organism. Our aim is to characterize the immune regulatory system in the simple purple sea urchin larva that will reveal causal interactions between the cells of the gut epithelium and mesodermal immunocytes during infection. The sea urchin larva has an optically transparent body that is made up of only a few thousand cells, yet a diverse array of immune regulators, receptors and effectors with close relationships to those of vertebrates are expressed. We first characterized four main subsets of larval immunocytes based on cellular behaviour such as phagocytosis of foreign particles and surveillance-like activity, as well as localization of known immune gene markers using *in-situ* hybridization. Massively parallel short sequencing and microarray were also used to identify candidate larval immune genes. We have also established a simple larval gut infection system using the marine bacterium *Vibrio diazotrophicus*, which results in the migration of pigmented immunocytes to the gut and the up-regulation of immune gene markers widely dispersed cellular compartments. Based on transcript prevalence measurements with this infection system, we identified additional candidate genes including IL-17 signalling factors and the transcription factor PU.1. *In-situ* hybridization and GFP reporter experiments are now underway to localize expression and to characterize the newly identified candidate genes for subsequent regulatory analyses. The examination of key genes within the framework of a basic immune circuit will provide anchor points for a more expansive gut immunity model.

13. Understanding soybean-induced gastroenteritis in salmonids using the rainbow trout (Oncorhynchus mykiss) enterocyte-like cell line RTgutGC

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Currently fish feed in salmonid aquaculture contains a high proportion of fishmeal derived from species of lower commercial value. This is a major issue facing aquaculture as fishmeal is costly and the source is limited, which could restrict aquaculture expansion, and might ultimately be unsustainable, necessitating a replacement. An attractive alternative is soybean meal because soybeans are an abundant source of protein similar in quality to fish meat. However, when soybean meal makes up a significant proportion of fish feed salmonids experience decreased growth and intestinal inflammation. These effects are attributed to antinutritional factors present in soybeans, with one group being lectins. The goal of this study is to begin to illuminate the etiology and immunology behind this intestinal inflammation by monitoring the effects of soybean components, on RTgutGC, a recently developed rainbow trout enterocyte-like cell line. Three lectins were tested in RTgutGC cultures. Soybean lectin (SBA) was not cytotoxic at 100 μg/ml for up to 31-day exposures. By contrast, red kidney bean (PHA-L) and wheat germ (WGA) lectins brought about morphological changes in the cells within 48 h and were cytotoxic after 7 days as evaluated with alamar Blue and CFDA AM. Since inflammation of the gut is commonly associated with gut barrier dysfunction, permeability assays were done using RTgutGC monolayers in inserts of 24-well Transwell bicameral plates. To date the lectins have not been seen to impair permeability but the assay needs further optimization. The effect of lectins on gene expression in RTgutGC is also being studied and the necessary tools for this are being developed. SBA and WGA induce the accumulation of beta 2 microglobulin as determined by western blotting. Also, the open reading frames of trout IL-8 and HMGB1, two proinflammatory cytokines, have been cloned for use in antibody production for testing protein expression.