EXPLORING THE ROLES OF *XENOPUS* MHC CLASS I-LIKE MOLECULES AND INNATE-LIKE T CELLS IN TUMOR IMMUNITY

Maureen Banach, Eva-Stina Edholm, PhD, Jacques Robert, PhD

Department of Microbiology & Immunology, University of Rochester Medical Center, Rochester, NY

The stimulation of natural killer T, so called innate-like (i) T cells, is a new therapeutic approach against some types of human cancers. However, the results are so far ambiguous because the conditions leading to either an anti- or pro-tumoral activity of these cells are only superficially understood. As iT cells recognize and interact with MHC class I-like molecules, it is also puzzling that many cancers highly express these molecules. To gain better insights into fundamental functions of iT and class I-like molecules, we use a comparative tumor immunity model in the amphibian *Xenopus*. Using RNA interference (RNAi) technology, we previously demonstrated that one of *Xenopus* MHC class I-like (*XNC*) genes, *XNC10*, is critical for the development and function of iT cells, expressing an invariant T cell receptor (TCR) α chain rearrangement – Vα6-Jα1.43. Furthermore, our data suggests that another iT cell subset, characterized by the invariant TCR rearrangement – Vα22-Jα1.32 is specifically involved in the immunity against *Xenopus* thymic lymphoid tumors. The intraperitoneal transplantation of *Xenopus* tumor cells into syngeneic tadpoles resulted in significant early infiltration of both Vα6-Jα1.43 and Vα22-Jα1.32, concomitant with the decrease of these subsets from spleen and thymus. We hypothesize that by impairing the tumor expression of *XNC10* and/or other *XNC* genes, the infiltration of specific iT cell subsets might be manipulated. To explore this possibility we are developing a reverse genetic approach with RNAi and three-component CRISPR/Cas9 system to generate transgenic animals deficient in Vα22 iT cells and tumor lines stably deficient in specific *XNC* genes.

THE *SpTransformer* GENE FAMILY HAS COMPLEX PATTERNS OF REPEATS, DUPLICATIONS, AND SHARED SEQUENCES CONSISTENT WITH GENOMIC INSTABILITY

Megan A. Barela Hudgell, Matan Oren & L. Courtney Smith

Department of Biological Sciences, George Washington University, Suite 6000, Science and Engineering Hall, 800 22nd St NW, Washington DC, 20052, USA

SpTransformer gene family encodes proteins with innate immune functions in the purple sea urchin. These genes have two exons a single diverse intron and are bracketed on both sides by GA and/or GAT short tandem repeats (STRs). Three clusters for a total of 15 genes are present in the genome sequence of the *S. purpuratus*. In this work an in-depth analysis was conducted to understand the sequence complexities of this gene family, its genomic structure, and to derive a hypothesis for the formation of the gene clusters. Results allowed for accurate naming of each gene, identification of the corresponding intron category, positions of stop codons, and relationships among the genes that have been used to infer their evolutionary relatedness. All genes share sequence similarity including flanking regions from the 5' STRs to the 3' STRs. The 5' end of 11 of 15 genes have two to three conserved short regions of similarity that are located 5' to the GA STRs. These regions may be indicative of short regulatory sites located at the 5' end of each gene. Two of the clusters that are thought to be allelic show differences in gene copy number and a region of ~11,000 bp with complete sequence dissimilarity. The complexity of this gene family suggests that regions with large numbers of repeats, duplications, shared sequences, and tight clustering could be due to, or the basis of, genomic instability. This may underpin the fast diversification rate that is commonly associated with immune genes.

FUNCTIONS AND APPLICATIONS OF CRISPR-CAS IMMUNE SYSTEMS

Rodolphe Barrangou

Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), together with associated sequences (cas) constitute the CRISPR-Cas system, which provides adaptive immunity against invasive elements in many bacteria and most archaea. In prokaryotes, CRISPR-Cas systems afford DNA-encoded, RNA mediated, nucleic acid targeting, using a diverse set of Cas nucleases that yield various cleavage outcomes, and carry out various biological functions. Recently, the CRISPR machinery has been repurposed to fuel the Cas9-enabled genome editing craze. Actually, the democratization of CRISPR-based technologies in the past three years hinges on the portability and functionalities of these molecular machines, and has revolutionized biology. I will look back on the historical milestones that have paved the way for the CRISPR era, and discuss the diverse applications that have impacted and continue to shape the field of Medicine, Ag, Food and Biotechnology. Importantly, there are numerous CRISPR-based applications in bacteria that span genotyping, phage resistance, immunization against invasive nucleic acids and antimicrobials that open avenues for the genesis of novel cultures and probiotics of high-potential for the food supply chain. Lastly, I will consider the impacts this transformative field has had on science and society, and discuss business implications of this disruptive technology.

KNOCKDOWN OF USF LEADS TO LONG-TERM CHANGES IN THE GENE EXPRESSION RESPONSE TO DNA DAMAGE

Kimberly Bellingham-Johnstun[1], John King[1], Kevin Gerrish[2], Pierre Bushel[3], and Michael Sikes[1]

[1]*Department of Biological Sciences, North Carolina State University, Raleigh, NC*
[2]*Molecular Genomics Branch, National Institute of Environmental Health Sciences, Durham, NC*
[3]*Computational Biology Branch, National Institute of Environmental Health Sciences, Durham, NC*

After receiving genotoxic insult, cells rapidly activate P53-dependent programs that pause the cell cycle and direct either DNA repair or apoptosis. Beyond P53, the long-term transcriptional programs that direct either cell recovery or senescence, depending on the success of DNA repair, are unclear. In this study, we used RNAi to investigate the potential of USF1 and USF2 stress-response transcription factors as directors of long-term DNA damage responses in the P53-deficient mouse B lymphocyte cell line, M12. Microarray analysis revealed 765 differentially expressed genes (≥1.50-fold change, n=3) in USF-depleted cells when compared with cells expressing a scrambled shRNA. In contrast, 7 days after cells were exposed to ionizing radiation, 2866 genes now showed altered expression in the USF-depleted cells. Microarray findings were recapitulated in separate biological replicates, and further confirmed for a panel of constitutively altered and IR-induced genes by RT-qPCR. In particular, USF-depletion led to the up-regulation of a number of genes critical for immune function and strongly linked to cancer development, including genes for the DNA mutator AID, the CD300a lipid receptor, the Ig kinases BLK and BLNK, and multiple members of the NFκB transcription factor family REL-A, REL-B, c-REL, IκBα and IκBδ. Together, our results provide the first evidence of a novel role for USF in regulating long-term response to DNA damage, while loss of USF results in overexpression of genes associated with B cell activation and tumorigenesis.

TGF-β2 DOWNREGULATES CONSTITUTIVE AND IFN-γ-INDUCED MHC SURFACE EXPRESSION ON EQUINE BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

Alix K. Berglund[1], Alexandra B. Grobman[2], Matthew B. Fisher[3], Lauren V. Schnabel[1]

[1]*College of Veterinary Medicine, North Carolina State University*
[2]*College of Agriculture and Life Sciences, North Carolina State University*
[3]*Joint Department of Biomedical Engineering, University of North Carolina – Chapel Hill and North Carolina State University*

Allogeneic mesenchymal stem cell (MSC) therapy for musculoskeletal diseases is currently hindered by recipient immune recognition of mismatched-major histocompatibility complex (MHC) molecules expressed on donor MSCs. Our hypothesis was that culturing equine MSCs with TGF-β2 would decrease constitutive MHC I and MHC II expression and block IFN-γ-induced MHC expression without affecting the viability or immunomodulatory properties of the cells. Bone marrow was aspirated from the sternum of twelve healthy horses and MSCs were isolated via Ficoll gradient centrifugation. MSCs were cultured with TGF-β2 before MHC I and MHC II surface expression was analyzed via fluorescent activated cell sorting (FACS). Cell yield and viability were measured at each passage. To determine if TGF-β2 blocked IFN-γ-induced MHC expression, untreated and TGF-β2-treated MSCs were stimulated with 1 ng/ml IFN-γ for up to 72 hours before FACS analysis. TGF-β2 treatment significantly reduced MHC I and MHC II surface expression on unstimulated MSCs and partially blocked IFN-γ-induced MHC I and MHC II surface expression. IFN-γ-induced MHC expression varied significantly between individual MSC donors, however. TGF-β2 treatment also improved cell yield at each passage and there was no significant difference between the viability of untreated and treated MSCs. TGF-β2 treatment did not significantly change the secretion of TGF-β1 from unstimulated or stimulated MSCs. These results demonstrate that TGF-β2 treatment has significant promise for reducing recipient immune recognition of MHC-mismatched molecules on allogeneic MSCs. Further work is needed to stabilize MHC expression in inflammatory conditions on MSCs and determine the immunogenicity of TGF-β2-treated MSCs.

A NON-PROTOTYPIC LEUKOCYTE IMMUNE-TYPE RECEPTOR (LITR) IS EXPRESSED IN RESPONSE TO VIRUS INFECTION IN THE CHANNEL CATFISH, *ICTALURUS PUNCTATUS*

Laura E. Blackmon, Melanie Wilson, and Eva Bengten

The University of Mississippi Medical Center, Jackson Mississippi

The channel catfish, *Ictalurus punctatus*, is a well-defined comparative model used to study innate and adaptive immunity. Leukocyte immune-type receptors (LITRs) are unique to teleosts and represent a large polymorphic and polygenic family of immunoregulatory receptors phylogenetically related to mammalian LRC-encoded proteins. Currently, LITRs are classified as type I or type II receptors with inhibitory or activating signaling capabilities, respectively. Some LITRs, defined by their reactivity with anti-LITR monoclonal antibody CC41, are distinct markers of a cytotoxic cell population which undergoes expansion during the antiviral response against Channel Catfish Virus (CCV). Recent genomic analyses have identified a new LITR subset, and in contrast to the previously described inhibitory type I and activating type II LITRs, members of this non-prototypic LITR subset lack typical D1-D2 immunoglobulin domains. Additionally, members of this subset are not CC41-reactive. To determine expression of this subset, G14D clonal catfish T cells and CCO (Channel Catfish Ovary) cells were subjected to CCV infection or stress conditions and harvested after challenge for RNA isolation, cDNA generation and subsequent PCR analysis. Interestingly, increased message expression of a member of this new subset, termed LITR-NP, is observed in G14D and CCO cells after infection with CCV. UV irradiation, heat shock or serum starvation, however, did not induce LITR-NP message expression. This expression pattern suggests that LITR-NP represents a functionally distinct LITR subset induced in virus infected cells and may serve as a target ligand for NK receptors in catfish.

THE FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*) IMMUNOGLOBULIN HEAVY CHAIN SUGGESTS THE IMPORTANCE OF CLAN III VARIABLE SEGMENTS IN REPERTOIRE DIVERSITY

Breanna Breaux[1], Thaddeus C. Deiss[1], Patricia L. Chen[1], Maria Paula Cruz-Schneider[2], Leonardo Sena[3], Margaret E. Hunter[4], Robert K. Bonde[4], Michael F. Criscitiello[1,5]

[1]*Department of Veterinary Pathobiology, Texas A&M University, College Station, TX*
[2]*Laboratory of DNA Polymorphism, Federal University of Pará, Belém, Pará Brazil*
[3]*Laboratory of Medical and Human Genetics, Federal University of Pará, Belém, Pará Brazil*
[4]*Wetland and Aquatic Research Center, U.S. Geological Survey, Gainesville, FL 32653 USA*
[5]*Department of Microbial Pathogenesis and Immunology, Texas A&M Health Science Center, College Station, TX*

Manatees are a vulnerable, charismatic sentinel species from the evolutionarily divergent Afrotheria. Manatee health and resistance to infectious disease is of great concern to conservation groups, but little is known about their immune system. To develop manatee-specific tools for monitoring health, we first must have a general knowledge of how the immunoglobulin heavy (IgH) chain locus is organized and transcriptionally expressed. Using the genomic scaffolds of the Florida manatee (*Trichechus manatus latirostris*), we characterized the potential IgH segmental diversity and constant region isotypic diversity. We then used 5' RACE PCR on peripheral blood leukocytes to perform the first Afrotherian repertoire analysis. The Florida manatee has low V(D)J combinatorial diversity (3744 potential combinations) and few constant region isotypes. They also lack clan III V segments, which may have caused reduced VH segment numbers. However, we found productive somatic hypermutation concentrated in the complementarity determining regions. In conclusion, manatees have limited IGHV clan and combinatorial diversity. This suggests that clan III V segments are essential for maintaining IgH locus diversity.

THE FLORIDA MANATEE (TRICHECHUS MANATUS LATIROSTRIS) T CELL RECEPTOR INTER-CHAIN AND INTER-SPECIES DIVERSITY

Breanna Breaux[1], Thaddeus C. Deiss[1], Maria Paula Cruz-Schneider[2], Leonardo Sena[3], Margaret E. Hunter[4], Robert K. Bonde[4], Michael F. Criscitiello[1,5]

[1]*Department of Veterinary Pathobiology, Texas A&M University, College Station, TX*
[2]*Laboratory of DNA Polymorphism, Federal University of Pará, Belém, Pará Brazil*
[3]*Laboratory of Medical and Human Genetics, Federal University of Pará, Belém, Pará Brazil*
[4]*Wetland and Aquatic Research Center, U.S. Geological Survey, Gainesville, FL 32653 USA*
[5]*Department of Microbial Pathogenesis and Immunology, Texas A&M Health Science Center, College Station, TX*

Manatees are a distinctive species in both biological niche and evolutionary position. They are also used as a measure of ecological fitness in coastal ecosystems. Due to their vulnerable conservation status, it is important to understand their immune robustness. We have previously described the immunoglobulin heavy chain locus of the Florida manatee (*Trichechus manatus latirostris*) and found limited antigen receptor diversity in this humoral arm of the adaptive immune system. We therefore investigated the loci of the other cellular arm of the adaptive immune system: the T cell receptor (TCR). We first annotated the genomic scaffolds of the *T. manatus* that contained the four TCR chains. Then we used 5' RACE PCR and PACBIO SMRT sequencing to capture the variability at each of the TCR chains to sample the repertoire of V(D)J rearranged transcripts in four individuals. We not only found a wide genomic segmental diversity, but varied combinatorial expression amongst all four individuals. We found that each chain utilizes a different strategy to create diversity that correlates to their individual functions. These results suggest that the manatee immune system may rely on cell-mediated immunity more than humoral immunity. Additionally, manatees are the first species from either Afrotheria or Xenarthra to have an in-depth characterization of all four TCR chains. Understanding the mammalian evolution of the TCR loci is lacking compared to the immunoglobulin loci. The inclusion of manatee in phylogenetic analyses will provide an essential link between marsupials and other eutherians to reveal evolutionary patterns for these complex loci.

TRANSCRIPTIONAL CONTROL OF A GUT-ASSOCIATED INFLAMMATORY RESPONSE IN A SIMPLE INVERTEBRATE DEUTEROSTOME

Katherine M. Buckley[1], Jonathan P. Rast[2]

[1]*Department of Biological Sciences, George Washington University, Washington, DC*
[2]*Department of Immunology and Department of Medical Biophysics, University of Toronto, Toronto, ON*

Sea urchin larvae are morphologically simple organisms that share an important genetic heritage with vertebrates, and provide an experimentally tractable system in which to characterize transcriptional control of the gut-associated immune response. In response to seawater exposure to the marine bacterium *Vibrio diazotrophicus*, larvae exhibit robust changes in cellular behavior and gene activity. Bacteria accumulate in the gut and later invade the body cavity where they are rapidly cleared by a coordinated response of phagocytic and granular immune cells. Peripheral immune cells migrate through the body cavity and accumulate at the gut. Analysis of RNA-Seq data from immune-challenged larvae reveals transcriptional changes in genes with homologs of important vertebrate immune factors, sea urchin-specific response genes and a set of novel genes with homologs that are widely distributed in bilaterians, but absent from vertebrates and ecdysozoans. The most acutely upregulated genes early in this response are two types of IL17 genes. Whole mount *in situ* hybridization and BAC-based fluorescent protein reporters indicate that these cytokines are expressed within gut epithelial cells. Perturbation of IL17 signaling results in reduced expression of *tnfaip3* (a negative feedback inhibitor of IL17), *nfkbiz* (a vertebrate IL17 target gene), *cebpα, cebpγ* and *soul1*. These results indicate that the highly regulated IL17 expression in the gut epithelium and signaling through IL17R1 form a central axis of larval gut-associated immunity. The morphological simplicity of this system provides a model to investigate system-wide molecular interactions at single-cell resolution and to characterize the distributed gene regulatory network that underpins immune response.

TRIM27.1 AND TRIM27-L FORM PROTEIN AGGREGATES NEAR MITOCHONDRIA

Lee K. Campbell and Katharine E. Magor

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

We are investigating the antiviral activity and oncogenic properties of TRIM27.1 and TRIM27-L in ducks. TRIM proteins have been shown to regulate viral infection in the cell by either direct viral restriction or modulation of signaling pathways. We previously showed that TRIM27-L upregulates and TRIM27.1 downregulates the interferon response after influenza infection. Interestingly TRIM27-L is found in ducks, but not in chickens, suggesting it might have a species-specific antiviral effect contributing to ducks' resistance to influenza infection, while chickens are susceptible. We have transiently expressed these TRIM proteins in primary duck fibroblasts, chicken fibroblasts (DF-1), chicken liver cells (LMH) and HeLa cells. Cells were stained with Thioflavin T to determine if proteins were forming organized aggregates. Using confocal microscopy we show TRIM27-L forms organized prion-like arrogates and TRIM27.1 instead appears to sequester around organized aggregates. TRIM27-L co-localizes with mitochondria while TRIM27.1 surrounds mitochondrial aggregates in duck fibroblasts and DF-1 cells. We will investigate whether either of the duck TRIM27s are interacting with MAVS to either enhance or inhibit the signaling response. Additionally, both duck TRIM27s cause oncogenic phenotypes when overexpressed in stable transfected DF-1 cells. We will assess stable clones to determine which pathways the duck TRIM27s are interacting with to cause tumorigenesis and loss of contact adhesion in these cells. Finally, we will generate inducible stable transfectants to assess the ability of TRIM27-L and TRIM27.1 to restrict influenza virus.

REQUIREMENT OF MONOCYTES/MACROPHAGES IN AMPHBIAN HOST DEFENSES AGAINST RANAVIRUS PATHOGENS

Francisco De Jesús Andino, Leta Yi, and Jacques Robert

Department of Microbiology and Immunology, University of Rochester Medical Center Rochester, NY 14642, USA

Monocytes/macrophages (Mθ), are both at the forefront of immune defenses against ranavirus pathogens and part of ranavirus infection strategies, which is unique among large DNA viruses. However, it is still unclear how critical these cells are for antiviral immune response and viral pathogenicity. To evaluate the role of Mθ during infection by the ranavirus FV3, we developed a Mθ depletion system in *Xenopus* by using clodronate-encapsulated liposomes. Clodronate treatment in adult *Xenopus* induces rapid death of cells with monocytic characteristics as determined by microscopy and flow cytometry. This results in dramatic impairment of host resistance to FV3 infection as shown by increased in viral load, viral dissemination and increased mortality. Interestingly, clodronate treatment is also effective in *Xenopus* tadpoles, resulting in an increase in apoptosis of peritoneal Mθ, associated with an increase in viral load and higher mortality level following FV3 infection. These data provide evidence of the critical roles of Mθ during FV3 infection.

GENOTYPE-SPECIFIC EXPRESSION OF *UNCLE FESTER* SUGGESTS A ROLE IN ALLORECOGNITION EDUCATION IN A BASAL CHORDATE

Daryl A. Taketa, Liviu Cengher, Delany Rodriguez, Adam D. Langenbacher, and Anthony W. De Tomaso

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

The basal chordate, *Botryllus schlosseri*, undergoes a natural transplantation reaction controlled by a single, highly polymorphic locus, called the *fuhc*. Allorecognition occurs at the tips of an extracorporeal vasculature, and two individuals that share one or both *fuhc* alleles are compatible, and the vessels will fuse, forming a parabiosis between the two individuals. In contrast, individuals with no common *fuhc* alleles between them will reject, an inflammatory reaction that results in melanin scar formation at the point of contact, blocking anastomosis. Fusibility is determined by sharing of a self-fuhc allele, reminiscent of the 'missing-self' mode of recognition utilized by vertebrate Natural Killer (NK) cells. Previous studies have demonstrated that two putative receptors, *fester* and *uncle fester*, are involved in this allorecognition response, and that fusion and rejection is due to integration of signals from these two proteins. *Uncle fester* plays a role in initiating the rejection response:. Here we report the existence of genotype-specific *uncle fester* expression levels, differing by up to 8-fold at the mRNA-level as confirmed by mRNA-Seq and qPCR. We also found that these changes had functional consequences: in incompatible pairings of genotypes with different expression levels of *uncle fester*, more points-of-rejection were present on the individual with higher *uncle fester* expression. These findings support previous conclusions that *uncle fester* levels modulate the rejection response, and offer an explanation for the variable strength of rejection phenotypes previously observed in *Botryllus schlosseri*. As fusion or rejection is determined by an integration of inputs from uncle fester, the long term maintenance of genotype specific expression is also evidence of an education process reminiscent of that which occurs in mammalian Natural Killer (NK) cells.

IMMUNOGENETIC FACTORS DRIVING FORMATION OF ULTRALONG CDR3 ANTIBODIES IN CATTLE

Thaddeus Deiss[1], Melissa Vadnais[2], Feng Wang[3], Pat Chen[1], Ali Torkamani[4], Waithaka Mwangi[5], Marie Paule Lefranc[6], Vaughn Smider[2], Michael Criscitiello[1]

[1]*Department of Veterinary Pathology, Texas A&M University-College of Veterinary Medicine, College Station, TX*
[2]*Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA*
[3]*California Institute for Biomedical Research, La Jolla, CA*
[4]*Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA*
[5]*Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS*
[6]*IMGT®, the international ImMunoGeneTics information system®, Laboratoire d'ImmunoGénétique Moléculaire, Institut de Génétique Humaine, CNRS, University of Montpellier Montpellier, France*

The repertoire of *Bos taurus* antibodies is characterized by a subset of heavy chains with variable domains VH encoding ultralong complementarity determining region CDR3. These ultralong CDR3 range from forty to over seventy amino acids in length and form a unique β-ribbon "stalk" and disulfide bonded "knob". Deep sequence analysis of the *B. taurus* heavy chain repertoire unveiled ultralong CDR3s were products of rearrangement between the longest VH and DH segments, IGHV1-1 and DH8. An eight nucleotide duplication at the 3' end of IGHV1-1, contributing the stalk, was observed as well as low variability in CDR1 and CDR2. This suggests diversity, thus antigen binding potential, of this subset lies primarily within CDR3. Deep sequencing also unveiled novel, potentially AID mediated, deletion events within the ultralong CDR3 subset in which only interior nucleotides of DH8 are removed. This leaves regions encoding the structurally relevant CPDG turn motif at the initiation of the knob and the alternating aromatic amino acids of the descending stalk untouched. The deletions, which range from 1-18 interior codons, were found to maintain an even number of cysteine residues throughout CDR3 in over 96% of sequences. Therefore the deletions serve not only to diversify the sequence content of the knob, but also cysteine positions within the knob, altering disulfide bonded loops. Hence, both germline and somatic genetic factors and processes appear to be involved in diversification of this structurally unusual bovine ultralong CDR3 repertoire.

LIVE AND LET DIE: A DEFENSE STRATEGY IN PLANTS

Yangnan Gu[1], Shui Wang[1,2], Sophie Zebell[1], Zizhen Liang[3], Byung-Ho Kang[3] and Xinnian Dong[1]

[1]*Howard Hughes Medical Institute-Gordon and Betty Moore Foundation, Department of Biology, PO Box 90338, Duke University, Durham, NC 27708, USA*
[2]*Development Center of Plant Germplasm Resources, College of Life and Environmental Sciences, Shanghai Normal University, Shanghai 200234, China*
[3]*School of Life Sciences, Centre for Cell and Developmental Biology and State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Hong Kong, China*

It has been a mystery how NB-LRR-mediated effector-triggered immunity (ETI) leads to PCD in plants in the absence of close homologs of caspases involved in pyroptosis during animal immune response-mediated by NLRs. Through a genetic screen for suppressors of a mutant with spontaneous PCD, *cpr5*, we found that the CKI-Rb-E2F cell cycle signaling pathway plays a key role in conferring PCD in plants during ETI mediated by both TIR-NB-LRR and CC-NB-LRR, two major classes of immune receptors in plants. Upon NB-LRR activation, CKIs are specifically released from association with the nuclear membrane protein CPR5, triggering E2F-mediated defense gene expression through hyperphosphorylation of Rb. Our recent work showed that CPR5 is a novel transmembrane nucleoporin. CPR5 associates with anchors of the NPC selective barrier to constrain nuclear access of signaling cargo and sequesters CKIs involved in ETI signal transduction. Upon activation by NB-LRRs, CPR5 undergoes an oligomer to monomer conformational switch, which coordinates CKI release for ETI signaling and reconfigures the selective barrier to allow significant influx of nuclear signaling cargo through the NPC. Consequently, these coordinated NPC actions result in simultaneous activation of diverse stress-related signaling pathways and constitute an essential regulatory mechanism specific for ETI/PCD induction.

MHC CLASS I LIKE RESTRICTED INNATE-LIKE T CELL LINEAGES WITH ANTI-MYCOBACTERIAL IMMUNE FUNCTIONS IN THE AMPHIBIAN *XENOPUS*

Eva-Stina Edholm, Kun Hyoe Rhoo and Jacques Robert

University of Rochester Medical Center, Department of Microbiology and Immunology, Rochester NY, USA

The biological relevance and evolutionary conservation of innate-like T (iT) cells have been strengthened by their discovery in the amphibian *Xenopus* where the tadpole T cell receptor repertoire (TCR) is dominated by six overrepresented invariant TCRα rearrangements indicative of 6 iT cell subset. We have previously identified one of these iT cell subset expressing the invariant Vα6Jα1.43 rearrangemnet that is restricted by the MHC class I-like molecule XNC10. Similar to CD1d restricted iNKT cells in humans and mice, iVα6 T cells in *Xenopus* are critical for early antiviral immune responses. Here, using a combination of RNAi loss-of-function by transgenesis targeting another *Xenopus* MHC class I-like gene, XNC4, we have identified a new distinct XNC4-dependent iT cell subset expressing one of the other 6 previously identified overrepresented iTCRs (TRAV45 joined to TRAJ1.14). Using XNC4 tetramers and adapting the CRISPR/Cas9-mediated gene editing technique to specifically disrupt the TRAJ1.14 segment thereby effectively ablating the iVα45 rearrangement, we show that this iVα45 T cell subset is not involved in antiviral immune response but is rather critical for immune response against *Mycobacterium marinum*. These data suggest that different iT cell lineages, restricted by distinct MHC class I-like molecules with functional specialization toward pathogens, play a prominent role in amphibian immune defense and as such may represent a more primordial immune cell type than previously thought.

AVIAN INFLUENZA IMMUNE EVASION – SPECIES-SPECIFIC NS1 PROTEIN INTERACTIONS IN HUMAN AND DUCK HOSTS

Danyel Evseev[1], Robert G. Webster[2] and Katharine E. Magor[1]

[1]*Department of Biological Sciences and Li Ka Shing Institute of Virology, University of Alberta, Edmonton, AB*
[2]*Division of Virology, Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN*

The ability of influenza viruses to block innate immune responses during establishment is crucial to their success in a host. The influenza non-structural protein 1 (NS1) is the main viral antagonist to innate immunity within host cells. In humans NS1 blocks the RIG-I signaling pathway of viral detection, through interactions with several pathway components. We are investigating whether NS1 proteins interfere with duck RIG-I signaling, because ducks are the reservoir hosts of influenza and can mount robust innate immune responses to highly pathogenic flu strains that kill chickens and humans. We compared the ability of NS1 from several low pathogenic and highly pathogenic influenza strains to interact with an essential co-activator of RIG-I, the TRIM25 protein, and show that different NS1 proteins interact with the human versus the duck orthologues, despite similar subcellular distribution patterns. Notably, NS1 proteins from a fatal human influenza isolate and from a closely-related avian isolate have different binding affinities for human TRIM25. We also show that none of the NS1 proteins bind to the signalling domain of RIG-I directly, in either species. Work is ongoing to test interactions with other RIG-I pathway components, to determine by mutagenesis the critical amino acid residues in NS1 that determine these interactions, and to perform *in vitro* infections with recombinant viruses. Knowing the sequence features of NS1 that contribute to human virulence is important for global surveillance and disease control. Comparing this to the function of NS1 in ducks will expand our understanding of the changes that occur when influenza jumps the species barrier.

This study is supported by CIHR

IMAGING FLOW CYTOMETRY-BASED HIGH-THROUGHPUT ASSAY IN EXAMINING TELEOST LEUKOCYTE IMMUNE-TYPE RECEPTOR MEDIATED PHAGOCYTOSIS

Chenjie Fei, Myron A. Zwozdesky, Dustin Lillico and James L. Stafford

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

Innate immune cell-mediated recognition, capture, and engulfment of large particulate targets such as bacteria is known as phagocytosis. This highly dynamic cellular process involves a series of steps including receptor-mediated target binding, phagocytic cup formation, pseudopod extension, and phagosome closure, which depend on distinct actin polymerization events. To further understand the phagocytic process in lower vertebrates, our research focuses on a polymorphic and polygenic immunoglobulin receptor family called Channel catfish (*Ictalurus punctatus*) leukocyte immune-type receptors (IpLITRs). Previous study using confocal microscopy revealed a distinct phagocytic phenotype where IpLITR1.1b expressing cells captured targets on their cell surface in stalled phagocytic cup-like structures. Quantitative analysis of phagocytic phenotypes using confocal microscopy, however, remains a daunting task. Here, we described a high-throughput method using imaging flow cytometry to distinguish internalized and surface-bound targets on individual cells with a high degree of accuracy and reproducibility. Specifically, extracellular binding yellow-green (YG) beads were stained by secondary Alex647-conjugated antibody shown in both green and red, while internalized YG beads were only shown in green due to inaccessible of the secondary antibody. Through the use of analysis features within the IDEAS® software combined with connected component masks, the accurate discrimination of surface-bound beads versus those internalized is clearly demonstrated. This novel method of differentiating surface-bound from internalized targets during phagocytosis provides more accurate determination of target-cell interactions that will assist in examination of the signalling events downstream IpLITR1.1b activation that leads to the distinct phagocytic phenotype.

DUCK RLR PATHWAY GENES ARE HIGHLY INDUCED BY H5N1 INFLUENZA

Ximena Fleming-Canepa[1], Lauren Canniff[1], David Tetrault[1], Jerry R. Aldridge, Jr.[2], Domingo Miranzo-Navarro[1], Robert G. Webster[2] and Katharine E. Magor[1]

[1]*Department of Biological Sciences, University of Alberta, Edmonton, AB*
[2]*Division of Virology, St. Jude Children's Research Hospital, Memphis, TN*

Retinoic acid inducible gene I (RIG-I) is upregulated in ducks during innate immune responses to avian influenza infection. However, the extent to which other MAVS pathway genes and interferon-stimulated genes (ISGs) are upregulated during this response is not known. Using reverse-transcription PCR we identified genes for RLR detectors (*DDX58* and *MDA5*), regulators (*TRIM25* and *RNF135*), signaling components (*MAVS* and *IRF7*), *IFNB* and downstream ISGs (*MX1*, *PKR*, *RSAD2*). We examined the relative abundance of these transcripts between 1 and 3 dpi with highly pathogenic A/Vietnam/1203/04 (H5N1) (VN1203) or low pathogenic strains A/mallard/BC/500/05 (H5N2) (BC500), A/mallard/BC/544/05 (H5N9) or NDV/P/mallard/Alberta 331/88 in lung and intestine of infected ducks known to be the sites of replication for these high and low pathogenic strains, respectively. Transcript abundance for genes in the MAVS signaling pathway is greatly increased in the lung during VN1203 infection, however transcript abundance changes little in the intestine during infection with low pathogenic strains. Induction is remarkably short-lived, with the transcript abundance peaking at 1 dpi and back to normal at 3 dpi. These results suggest that the innate immune response to VN1203 in lungs is rapid and robust, while the response to low pathogenic virus in intestine is weak. This may help explain how ducks survive highly pathogenic strains that can kill other host species, and yet permit replication of low pathogenic strains.

Ig ISOTYPE BINDING BY POLYMERIC Ig RECEPTORS (PIGRS) IN *XENOPUS*

Flowers E.M.[1], Neely H.R.[2], Ohta Y.[1], and Flajnik M.F.[1]

[1]*Department of Microbiology and Immunology, University of Maryland Baltimore School of Medicine, Baltimore, MD, USA*
[2]*Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA, USA*

A single population of antigen presenting cells (APCs) called XL (*Xenopus laevis*) cells has been described in the *X. laevis* spleen. After immunization, XL cells migrate to the internal perimeter of the B cell zone and bear native antigen on their surface, as well as IgM, the IgA orthologue IgX, and the IgG orthologue IgY, presumably in complex with the antigen. It is unknown which Fc Receptors (FcR) are involved in immune complex acquisition by XL cells. Two copies of polymeric Ig receptor (pIgR) family, *xlPIGR* and *xlPIGR2*, are found in the *Xenopus* genome. In general, pIgR is the oldest identifiable FcR and transports mammalian IgM and IgA across mucosal epithelial cells. *xlPIGR* is the orthologue of mammalian pIgR, is expressed at high levels in intestine and lung, and the encoded receptor was shown previously to interact with *Xenopus* J-chain. We found that *xlPIGR2* is highly expressed by XL cells and thus it could be involved in antigen acquisition, processing, and presentation. Using stably transfected 293 cells we investigated Ig isotype binding by xlPIGR and xlPIGR2. Consistent with prior work, we showed that xlPIGR bound IgM, and further demonstrated its failure to bind to IgX or IgY. IgX is a polymeric mucosal antibody, but it seems to assemble without J-chain, which may explain its lack of binding to xlPIGR. xlPIGR2 bound IgM and IgX, but not IgY, suggesting that xlPIGR2 binding is J chain-independent and that it may be involved in immune complex binding and antigen handling by XL cells.

IDENTIFICATION OF NOVEL IMMUNE MEDIATORS IN THE TRANSCRIPTOME OF *STRONGYLOCENTROTUS PURPURATUS* COELOMOCYTES

Mei-Chen Liu[1], Wen-Yun Liao[1], Katherine M Buckley[4,5,6], Shu Yuan Yang[1,2,7], Jonathan P. Rast[4,5,6], Sebastian D. Fugmann[3,8,9]

[1]Department of Biomedical Sciences, [2]Division of Biochemistry, Molecular and Cellular Biology, Graduate Institute of Biomedical Sciences, [3]Division of Microbiology, Graduate Institute of Biomedical Sciences, Chang Gung University, Tao-Yuan, Taiwan
[4]Biological Sciences, Sunnybrook Research Institute, Toronto, Ontario, Canada
[5]Department of Medical Biophysics, [6]Department of Immunology, University of Toronto, Toronto, Ontario, Canada
[7]Department Of Pathology, [8]Department of General Surgery, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan
[9]Chang Gung Immunology Consortium, Chang Gung Memorial Hospital and Chang Gung University, Kwei-Shan District, Tao-Yuan, Taiwan

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 initiated a RNAseq-based analyses of the transcriptomes of the four most abundant coelomocyte types. We discovered a large number of genes expressed specifically in distinct coelomocyte types, but we also identified numerous novel transcripts for which the corresponding genes have not been annotated yet. This includes a novel family of AID/APOBEC-like cytidine deaminases. Interestingly, the AID/APOBEC deaminases were previously thought to be exclusive to vertebrates, but our extended database searches now reveal the presence of numerous genes encoding such enzymes throughout the invertebrate world. Taken together, the findings from our coelomocyte transcriptome dataset are in line with the emerging concept that the adaptive immune system including factors that are now fount to be central to vertebrate adaptive immunity, appeared much more gradually than previously anticipated.

This work was supported by Chang Gung Memorial Hospital grants (CMRPD1C0242, CMRPD1F0421, CORPD1F0051) and a Ministry of Science and Technology grant (102-2320-B-182 -001 -MY3) to SDF.

CHARACTERIZING THE INFLAMMATORY CAPACITY OF RESIDENT CELLS OF THE INTERVERTEBRAL DISC

Matthew Guerreiro, Bridgette Laird, Diane E Gregory, Stephanie J DeWitte-Orr

Wilfrid Laurier University 75 University Ave W, Waterloo, Ont, Canada, N2L 3C5

Low back pain poses a significant strain on our healthcare system with an annual price tag in excess of $100 billion per year in North America alone. Intervertebral disc (IVD) herniation leads to mechanical compression of the nerve roots and/or inflammation caused by the innate immune response, causing low back pain. Herniation is defined as a tear in the outer annulus fibrosus (AF) resulting in the migration of the inner nucleus pulposus (NP) through the AF. Macrophages are known to migrate to the site of damage, exacerbating inflammation; however, very little is known of the role of resident IVD cells in starting the initial wave of inflammation and recruiting macrophages to the site of damage. The present research aims to fill this void by studying resident AF and NP cells derived from rat tail IVDs; first by developing primary cell cultures for each cell type, then characterizing their innate immune sensor repertoire and finally studying how these cells respond to inflammatory stimuli, both pathogen and host derived. Understanding the progression of the innate immune response will allow treatments targeting IVD cell sensors in order to limit low back pain.

REANALYSIS OF THE EVOLUTION OF TUMOR NECROSIS FACTOR SUPERFAMILY IN VERTEBRATES

Ryan D. Heimroth and Irene Salinas

Center for Evolutionary and Theoretical Immunology (CETI), Department of Biology, University of New Mexico, Albuquerque, NM

Tumor necrosis factor superfamily ligand and receptor families (TNFSF) are an ancestral group of cytokines that play a vital role in many cellular functions such as cell differentiation and activation, and apoptosis. Additionally, TNFSFs are crucial for lymphoid tissue development and organization in mammals. Previous work has postulated that the progressive organization of lymphocytes observed in vertebrate evolution is a result of the diversification of TNFSF members. In order to revisit this hypothesis, we have reanalyzed the presence of TNFSF genes in newly available genomes from teleosts, coelacanth, amphibians, reptiles, birds and mammals. Additionally, seven lungfish transcriptomes were screened using both BLAST searches and HMM protein homology searches. Bayesian phylogenetic analysis was performed using the conserved TNF homology domain and the conserved TNFRSF motifs. Our results show expansions of TNF ligands in the South American lungfish (*Lepidosiren paradoxa*) with known functions in cell death and apoptosis in mammals but not of members involved in lymphoid tissue organization. African lungfish (*Protopterus sp)*, in turn, express greater number of TNFSF genes with lymphocyte organization function. RT-qPCR analyses of 7 different *P. dolloi* tissues found a high constitutive expression of TNFRSF11A and IL7R in the nose, a region where lymphoid aggregation is known to occur in this species. We are currently performing comparative RNAseq analysis of laser-capture microdissected nasal lymphoid aggregates from *P. dolloi* as well as mammalian lymph nodes and Peyer's patches. Our findings will unveil the molecular drivers of lymphocyte aggregation and confirm or reject the TNFSF evolutionary hypothesis.

IDENTIFYING CYTOPLASMIC NUCLEIC ACID SENSORS, DHX9 AND DDX3, IN RAINBOW TROUT

Shanee L. Herrington-Krause, Sarah J. Poynter, Stephanie J. DeWitte-Orr

Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, CANADA

Innate immunity constitutes the first line of defense against virus infections. Viruses produce nucleic acids, both RNA and DNA, during genome replication and transcript synthesis. These nucleic acids are foreign to the cell and are sensed by pattern recognition receptors, based on their type (RNA or DNA), their strandedness (ss or ds) and their location (endosomal, extracellular or cytoplasmic). When a viral nucleic acid is in the wrong compartment (ex. dsDNA in the cytoplasm), they are sensed by pattern recognition receptors (PRRs) which activate signalling cascades that culminate in the production of type I interferons (IFNs) and the induction of an antiviral state. Cytoplasmic RNA sensors, such as RIG-I and MDA5, have begun to be characterized in several fish species, but almost nothing is known of cytoplasmic DNA sensors (CDSs) in fish. To this end, two CDSs were cloned from the rainbow trout gonadal cell line RTG2. Both CDSs are ATP dependent RNA helicases that in mammals function as innate immune sensors to initiate an antiviral state via the IFN pathway during a virus infection. In this study the novel rainbow trout DHX9 and DDX3 sequences were compared to known vertebrates sequences to identify conserved protein domains, intron/exon structures and phylogeny. Their responses to viral infection are currently under investigation. Knowledge of CDSs in rainbow trout will aid in a better understanding of innate antiviral immunity in this commercially and economically important fish species.

THE CHICKEN-*SALMONELLA* ENIGMA: DECIPHERING THE INTERACTION BETWEEN DIET, MICROBIOME, AND HOST MUCOSAL GENE EXPRESSION IN THE RESPONSE TO *SALMONELLA* INFECTION IN CHICKENS.

Rebecca-Ayme Hughes[1,2], Rizwana Ali[2], Mary Mendoza[2], Hosni Hassan[2], and Matthew Koci[2]

[1]*Department of Chemistry, North Carolina State University, Raleigh NC*
[2]*Prestage Department of Poultry Science, North Carolina State University, Raleigh NC*

Salmonellosis is a leading cause of foodborne illness worldwide. In the US it is estimated to cause over 1 million illnesses and costs the US economy over $2 billion annually. Many of these illnesses have been linked to the consumption of poultry products, as they can be asymptomatic carriers. While there are numerous efforts to produce anti-*Salmonella* vaccines with the goal of reducing *Salmonella* loads through immunity, it is unclear how effective these programs are. In order to develop effective methods to control *Salmonella* carriage by chickens, we need a better understanding of how *Salmonella* affects the ecology of the intestine, and how the host mucosal immune system responds in kind. To begin to understand these complex interactions, we used prebiotics, probiotics, and modified live *Salmonella* vaccines to alter the intestinal microbiome and then investigated how this affected *Salmonella* colonization of the intestine, invasion of the host, and changes in gene expression of mucosal immune tissue. The results of these experiments demonstrate that while *S*. Typhimurium was more successful than *S*. Enteritidis, regardless of treatment group, *Salmonella* is not able to invade and persist in examined tissues. The treatments did affect the makeup of the host microbiota, but these differences did not result in significant differences in duration of *Salmonella* found in the cecum. Furthermore, changes in host mucosal immune tissue gene expression demonstrated differences, these differences were subtle and provide further evidence that the chicken does not regard *Salmonella*, specifically *S*. Typhimurium and *S*. Enteritidis, as a pathogen.

DEFINING THE MECHANISMS BY WHICH TRIM9, A UBIQUITIN LIGASE, MEDIATES INNATE IMMUNE CELL FUNCTION

Amanda N. Kortum, Debra Tokarz, Jeffrey A. Yoder

Department of Molecular Biomedical Sciences and Comparative Medicine Institute, North Carolina State University College of Veterinary Medicine, 1060 William Moore Drive, Raleigh, NC 27607 USA

The persistence of an immune response can contribute to the development of disease states, amplifying the extent of tissue damage or perpetuating the disease condition. Damaging inflammatory states are often characterized by high numbers of macrophages and neutrophils. Studying the cellular mechanisms required for phagocyte migration to sites of inflammation will elucidate how these pathways could be therapeutically targeted for disruption during persistent, harmful inflammation. Preliminary data identifies a novel role for the E3 ubiquitin ligase Tripartite Motif Containing 9 (TRIM9) as a critical mediator of macrophage motility in the zebrafish model. This is in agreement with the well-defined role TRIM9 plays in mediating neuronal axon migration in mammals. While several members of the TRIM family have known regulatory roles in the NFκB and interferon signaling pathways through interactions with a wide range of substrates, the functional roles of TRIM9 in immune cells are currently undefined. We hypothesize that TRIM9 plays a critical role in multiple immune functions by mediating a novel phagocyte-specific ubiquitin pathway. For this work, we will determine which phagocyte immune functions, such as migration and phagocytosis, are mediated by TRIM9 and begin to define the TRIM9 protein interactome. This research will begin to define the immunologically relevant cellular and molecular processes mediated by TRIM9 and its protein network in mammalian phagocytes and contribute to an area of research that has great potential for producing targeted therapeutics for conditions where persistent inflammatory states contribute to the disease process.

IMMUNE ROLES OF AMPHIBIAN (*Xenopus laevis*) TADPOLE GRANULOCYTES DURING FROG VIRUS 3 RANAVIRUS INFECTIONS

Daphne V. Koubourli, Amulya Yaparla, and Leon Grayfer

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

Infections by Frog Virus 3 (FV3) and other ranaviruses (RVs) are contributing to the amphibian decline, but the mechanisms controlling anuran tadpole susceptibility and adult frog resistance to RVs, including the roles of polymorphonuclear granulocytes (PMNs) during anti-FV3 responses, remain largely unknown. Since amphibian kidneys represent an important FV3 target, the inability of amphibian (Xenopus laevis) tadpoles to mount effective kidney inflammatory responses to FV3 is thought to contribute to their susceptibility. Here we demonstrate that a recombinant X. laevis granulocyte colony-stimulating factor (G-CSF) generates PMNs with hallmark granulocyte morphology. Tadpole pretreatment with G-CSF prior to FV3 infection reduces animal kidney FV3 loads and extends their survival. Moreover, G-CSF-derived PMNs are resistant to FV3 infection and express high levels of TNFα in response to this virus. Notably, FV3-infected tadpoles fail to recruit G-CSFR expressing granulocytes into their kidneys, suggesting that they lack an integral inflammatory effector population at this site.

STAYING ALIVE! SIGNAL PROCESSING BY NF-κB PROTECTS NKT CELLS FROM TNFR1-TRIGERED DEATH

Amrendra Kumar[1,2], Laura E. Gordy[2], Jelena S. Bezbradica[2,3], Aleksandar K. Stanic[2,4], Mark R. Boothby[2], Luc Van Kaer[2] & Sebastian Joyce[1,2]

[1]*Veterans Administration Tennessee Valley Healthcare System, Nashville, Tenessee, USA*
[2]*Department of Pathology, Microbiology & Immunology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA*
[3]*The Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK*
[4]*Department of Obstetrics and Gynecology, University of Wisconsin-Madison, Madison, Wisconsin, USA*

Semi-invariant natural killer T (NKT) cells are innate-like lymphocytes with immunoregualtory properties. NKT cell survival during development requires signal by activated RelA/NF-κB. Yet the upstream signal(s) integrated by NF-κB in developing NKT cells remains incompletely defined. We show that the introgression of *Bcl2l1* transgene into NF-κB signalling deficient IκBΔN transgenic mouse rescues NKT cell development and differentiation in this mouse model. We reasoned that NF-κB activation was protecting developing NKT cells from death signals emanating either from high affinity agonist recognition by the T cell receptor (TCR) or from a death receptor, such as tumour necrosis factor receptor 1 (TNFR1) or Fas. Surprisingly, the single and combined deficiency in PKC-θ or CARMA-1—the two signal transducers at NKT TCR proximal signalling node—only partially recapitulated the NKT cell deficiency observed in $I\kappa B\Delta N^{tg}$ mouse. Accordingly, introgression of the Bcl-x_L transgene into PKC-θ null mouse did not rescue NKT cell development. Instead, the introgression of the TNFR1-null mutation, but not the Fas function-disrupting *lpr/lpr* mutation, into NF-κB -signalling deficient $I\kappa B\Delta N^{tg}$ mice rescued NKT cell development. Hence, we conclude that signal integration by NF-κB protects developing NKT cells from death signals emanating from TNFR1, but not from NKT TCR or Fas.

MR1 RECOGNITION BY HUMAN γδ T CELLS

Jérôme Le Nours [1,2], Nicholas A. Gherardin[3,4], Victoria A. Hughes[1,2], Andrew N. Keller[1,2], Florian Wiede[1], Benjamin S. Gully[1,2], Richard Berry[1,2], Maria L Sandoval-Romero [1], Shihan Li[3,4], Sidonia B.G. Eckle[3], Alexandra J. Corbett[3], Ligong Liu[5,6], David P. Fairlie[5,6], Tony Tiganis[1], James McCluskey[3], Daniel G. Pellicci[3,4], Adam P. Uldrich[3,4], Dale I. Godfrey[3,4] & Jamie Rossjohn[1,2,7]

[1]*Infection and Immunity Program and Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria 3800, Australia*
[2]*Australian Research Council Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton, Victoria 3800, Australia*
[3]*Department of Microbiology & Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne, Parkville, Victoria 3010, Australia*
[4]*Australian Research Council Centre of Excellence in Advanced Molecular Imaging, University of Melbourne, Parkville, Victoria 3010, Australia*
[5]*Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland 4072, Australia*
[6]*Australian Research Council Centre of Excellence in Advanced Molecular Imaging, University of Queensland, Brisbane, Queensland 4072, Australia*
[7]*Institute of Infection and Immunity, Cardiff University School of Medicine, Heath Park, Cardiff CF14 4XN, UK*

The T lymphocytes repertoire is divided into two major lineages, αβ and γδ T cells, that are defined by their T cell receptor (TCR) gene-segment usage. The MHC-like molecule MR1 presents vitamin-B derivatives to mucosal-associated invariant T-cells. Using MR1 tetramers, we characterized a population of MR1-restricted human γδ T cells that included phenotypically diverse Vγ8-Vδ1, Vγ9-Vδ1 and Vγ8-Vδ3 subsets, all of which exhibited MR1 autoreactivity, independent on the nature of the bound ligand. The structure of a γδTCR-MR1-antigen complex showed the γδTCR docked in a highly unusual manner that starkly contrasted all other TCR complex structures. The γδTCR bound perpendicular to MR1, clamping around one end of the MR1 antigen-binding cleft. Contacts were mediated exclusively by the TCR γ-chain and MR1, which included residues from the γδTCR constant domain. Accordingly, we define MR1 as a target for γδ T-cells and show that the γδTCR constant domain can contribute directly to antigen specificity. Our findings reshape our understanding of TCR recognition determinants and γδT-cells.

INDIRECT ACTIVATION OF DENDRITIC CELLS DURING *STREPTOCOCCUS SUIS* SEROTYPE 2 INFECTION IN A MURINE MODEL

Corinne Letendre[1], Jean-Philippe Auger[2], Marcelo Gottschalk[2], Jacques Thibodeau[3], Mariela Segura[1].

[1]*Laboratory of Immunology, Faculty of Veterinary Medicine, University of Montreal, St-Hyacinthe, QC, Canada*
[2]*Laboratory of* Streptococcus suis*, Faculty of Veterinary Medicine, University of Montreal, St-Hyacinthe, QC, Canada*
[3]*Laboratory of Molecular Immunology, Department of Microbiology, Infectiology and Immunology, University of Montreal, Montreal, QC, Canada*

Streptococcus suis is an important swine pathogen and emerging zoonotic agent, for which there is still no effective vaccine available. This bacterium causes acute systemic infections characterized by high levels of pro-inflammatory mediators. Inflammatory conditions such as sepsis can lead to indirectly activated DCs with impaired antigen presentation capacities. Interestingly, *S. suis* is known to modulate dendritic cell (DC) functions and interfere with $CD4^+$ T cell activation. Here, we report the antigen presentation profile of splenic DCs during a systemic infection in a murine model. Splenic DCs obtained from *S. suis*-infected mice showed expression profiles of CD86/MHC-II and transcription levels of CIITA and MARCH1/8 that were compatible with indirect activation of DCs. Moreover, the IL-12 production capacity of these cells was impaired in an *ex vivo* assay, while production of other cytokines remained unaffected. Infected splenic DCs also failed to induce the production of the Th1-derived cytokines IL-2 and TNF-alpha in an *ex vivo* antigen-specific $CD4^+$ T cell presentation assay. Hence, we conclude that *S. suis* infection leads, at least partly, to indirect activation of DCs. These cells have an impaired MHC-II-restricted antigen presentation capacity and produce limited amounts of IL-12, the main Th1-polarizing cytokine. As Th1-type responses have been associated with host protection against *S. suis*, this mode of DC activation might interfere with the development of an appropriate response. This study highlights the potential consequences of inflammation on the type and magnitude of the immune response elicited by a pathogen.

LYMPHOCYTOPENIA AND SPLENIC LYMPHOID DEPLETION IN CAPTIVE ADULT MALES OF A SEMELPAROUS MARSUPIAL SPECIES, THE RED-TAILED PHASCOGALE *(PHASCOGALE CALURA)*

Corinne Letendre, Ethan Sawyer, Julie M. Old

School of Science and Health, Western Sydney University, Penrith, NSW, Australia

The red-tailed phascogale is a small endangered Australian marsupial. Like in some other dasyurid species, males are semelparous, which means they die shortly after reproducing. This total male mortality is stress-related and associated with suppression of the immune and inflammatory reactions. In captivity, males have a longer lifespan than they do in the wild but become infertile after their first breeding season. However, it remains unknown whether they also exhibit a stress response and how this affects their immune function. The aim of this study is to investigate the immune profile of captive adult males, compared to juvenile males and their female counterparts. Blood and spleens were opportunistically collected from different age-sex groups (n=6 per group) in a captive breeding colony, between December 2016 and January 2017. Hematological results reveal that adult males exhibit a mild lymphocytopenia and neutrophilia compared to other age-sex groups, which is consistent with a stress response. Histopathological analysis of spleen sections was performed and the size and cellularity of the splenic lymphoid white pulp (periarteriolar sheaths, follicles and marginal zones) were evaluated on a 4-grade scale by masked examination. Lymphoid depletion was observed in adult male spleens, with most individuals showing severe follicular atrophy. These results suggest that the immune capacities of red-tailed phascogale males might be impaired as they age in captivity. Further studies will be needed to assess their ability to mount functional immune responses. Conservation programs and research on health and disease of those endangered marsupials will benefit from these new findings.

EXAMINING TELEOST LEUKOCYTE IMMUNE-TYPE RECEPTOR MEDIATED INDUCTION OF PHAGOCYTIC TENTACLES

Dustin Lillico, James Stafford

Department of Biological Sciences, University of Alberta, Edmonton, Alberta, T6G 2R3

Fundamental for nutrient acquisition in primitive unicellular organisms such as amoeba, phagocytosis has evolved into a complex and important component of innate immunity and tissue homeostasis in all multicellular organisms. To further understand the phagocytic process in vertebrates, my research focuses on characterizing the channel catfish (*Ictalurus punctatus*) leukocyte immune-type receptor (IpLITR) proteins. In the present study I used IpLITR-transfected rat basophilic leukemia (RBL)-2H3 cells to further compare the phagocytic behavior mediated by IpLITR 2.6b (classical ITAM-dependent pathway) with the novel ITAM-independent pathway evoked by the functionally versatile IpLITR 1.1b protein. The specific goal of this study was to address the hypothesis that IpLITR 1.1b uniquely regulates the deployment of membrane structures called filopodia that may participate in the early stages of phagocytosis. Specifically, using live-cell video imaging of cells co-transfected with LifeAct and high-resolution SEM, I show that IpLITR 1.1b-expressing cells generate F-actin dense filopodia-like protrusions during the early stages of the phagocytic process. In addition, these structures retract back towards the cell after target contact to secure captured microspheres on the cell surface. This activity was then followed by distinct F-actin polymerization dynamics leading to the formation of phagocytic cups and in some cases the eventual engulfment of the microspheres. Overall, these results support the hypothesis that IpLITR 1.1b regulates an alternative phagocytic pathway that is functionally distinct from the prototypical pathway observed for IpLITR 2.6b. The molecular mechanisms that regulate the generation of IpLITR-induced phagocytic tentacles are currently being investigated.

AID/APOBEC –LIKE CYTIDINE DEAMINASES ARE ANCIENT INNATE IMMUNE MEDIATORS IN INVERTEBRATES.

Mei-Chen Liu[1], Wen-Yun Liao[1], Katherine M Buckley[4,5,6], Shu Yuan Yang[1,2,7], Jonathan P. Rast[4,5,6], Sebastian D. Fugmann[3,8,9]

[1]Department of Biomedical Sciences, [2]Division of Biochemistry, Molecular and Cellular Biology, Graduate Institute of Biomedical Sciences, [3]Division of Microbiology, Graduate Institute of Biomedical Sciences, Chang Gung University, Tao-Yuan, Taiwan
[4]Biological Sciences, Sunnybrook Research Institute, Toronto, Ontario, Canada
[5]Department of Medical Biophysics, [6]Department of Immunology, University of Toronto, Toronto, Ontario, Canada
[7]Department Of Pathology, [8]Department of General Surgery, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan
[9]Chang Gung Immunology Consortium, Chang Gung Memorial Hospital and Chang Gung University, Kwei-Shan District, Tao-Yuan, Taiwan

Vertebrate immune systems utilize targeted alterations of nucleotide sequences within both host and pathogen as powerful defense mechanisms. During innate and adaptive immune responses, activation induced cytidine deaminase (AID)/apolipoprotein B editing complex (APOBEC) cytidine deaminases initiate such processes. Their appearance in evolution coincides precisely with the emergence of adaptive immunity in vertebrates. Here we now report the discovery of novel genes in two invertebrates, *Strongylocentrotus purpuratus* and *Lingula anatina*, encoding proteins with striking similarities of amino acid sequence and enzymatic activities to vertebrate AID/APOBECs. Their expression is highest in tissues with constant intimate interactions with microbes, and can be induced upon pathogen challenge. Our findings strongly suggest that cytidine deamination represents an ancient innate immune mechanism dating back to protostomes.

This work was supported by Chang Gung Memorial Hospital grants (CMRPD1C0242, CMRPD1F0421, CORPD1F0051) and a Ministry of Science and Technology grant (102-2320-B-182 -001 -MY3) to SDF.

EVOLUTIONARY CONSERVATION OF CD4 AS A RECEPTOR FOR IL-16: PRELIMINARY EVIDENCE FROM *XENOPUS LAEVIS*.

Taylor Uccello, Gregory D. Maniero, Jacob Gillis

Stonehill College, 320 Washington Street, North Easton, MA 02357

IL-16 is a pro-inflammatory cytokine shown to attract and activate T cells by binding to CD4 outside of the MHC-binding domain. The role of CD4 as a receptor for IL-16 has been conserved throughout vertebrate evolution and fragments of recombinant human IL-16 (rhIL-16) can stimulate a variety of mammalian T cells.

Xenopus represents a well-established model for the study of the evolution and conservation of vertebrate immunity. The gene for CD4 is known in *Xenopus* but little characterization of this molecule or of $CD4^+$ T cells, has been performed largely due to the absence of an anti-*Xenopus* CD4 antibody. The *Xenopus* CD4 gene lacks the sequence for the canonical FLXK MHC-binding site described on mammalian T cells but encodes an IL-16-binding site. Conversely, the gene for *Xenopus* IL-16 encodes a conserved CD4-binding sequence.

Xenopus lymphocytes labelled with rhIL-16 and separated on a magnetic column yielded a fraction of $IL\text{-}16^+$ lymphocytes similar to that of $CD8^-$ cells. RT-PCR revealed that *Xenopus* lymphocytes cultured with rhIL-16 upregulate MHC class II mRNA, a phenomenon described in mammals. Peritoneal injection of rhIL-16 resulted in a substantial increase in lymphocytes in the coelom, suggesting that IL-16 attracts lymphocytes in frogs as it does in mammals. $RhIL\text{-}16^+$ lymphocytes were detected, although at low levels, by immunohistochemistry and flow cytometry.

We conclude that *Xenopus* lymphocytes bind and are activated by IL-16, most likely through interactions with CD4. We intend to exploit this interaction to purify *Xenopus* CD4 and $CD4^+$ cells and produce an anti-*Xenopus* CD4 antibody.

WIDESPREAD STRUCTURAL AND CODING SEQUENCE VARIATION HIGHLIGHT RAPID AND ONGOING EVOLUTION OF ZEBRAFISH IMMUNE GENES

Sean C. McConnell[1], Kyle M. Hernandez[2], Jorge Andrade[2], Jill L.O. de Jong[1]

[1]*Section of Hematology-Oncology and Stem Cell Transplant, Department of Pediatrics, The University of Chicago, 900 East 57th St, Chicago, IL 60637, USA*
[2]*Center for Research Informatics, The University of Chicago, 5751 S. Woodlawn Avenue, Chicago, IL 60637, USA*

Zebrafish excel as a model organism for developmental biology and disease modeling. Traditionally, laboratory zebrafish have been maintained as outbred populations with high genetic variability. Our recent work has examined the core MHC locus in zebrafish, where we found alternative pathways of antigen processing and presentation genes that are separated by 500 million years of evolution. Here we performed high-coverage genomic sequencing for two clonal lines of zebrafish, and one partially inbred zebrafish, to uncover additional sources of immune gene variation throughout these genomes. Pathway analysis identified immune genes as highly enriched among genes under positive selection, or associated with structural variation. Overall, zebrafish genomes are enriched by approximately 5 fold higher levels of variation compared with humans, including SNVs, small indels, and structural variants. Such variation affects additional MHC loci in zebrafish, as well as many other innate immunity genes including the NLR genes. Strikingly, the number of variants present on one arm of zebrafish chromosome 4 (including the bulk of NLR genes, with haplotypic variants covering 10-20Mb) is nearly what is found in an entire human genome. This disproportionately high variation likely impacts phenotypic traits, particularly those related to immune function. Experimental approaches dependent on known sequences are also highly affected, for example CRISPR/Cas9 gene editing. Genomic sequences for clonal zebrafish lines help to highlight, define, and characterize this rich variation, advancing functional studies in this vertebrate model, and providing insights into the evolution of vertebrate immunity.

Funding: Postdoctoral award from the Chicago Biomedical Consortium (SCM).

OPSONIC ACTIVITY AND IMMUNOHISTOCHEMICAL LOCALIZATION OF SEC3, A COMPLEMENT COMPONENT C3-LIKE PROTEIN FROM *SWIFTIA EXSERTA*

Menzel, LP, and Bigger, CH

Department of Biological Sciences, Florida International University

The third Complement component (C3) is the central protein of the complement cascade linking the three activation pathways (the antibody-dependent classical pathway, the lectin-dependent pathway, and the alternative pathway) with the lytic cascade. C3 is an evolutionarily ancient molecule involved with host defense: C3 genes have been found in many metazoan phyla. In fact our lab published the first report of C3 from a cnidarian, the octocoral *Swiftia exserta*.

Functional studies of complement cascade activity began with Bordet and the burgeoning field of immunology, investigating mammalian complement systems. These studies include the end-of-cascade lytic pores developed by the membrane attack complex (MAC), opsonic functions of C3b (a fragment of C3 covalently bound to targets via a thiol-ester bond), and chemo-attraction assays of C3a (as well as C4a and C5a fragments from C4 and C5, respectively). Opsonization is the labeling of particles for phagocytosis, causing opsonized particles to be ingested faster than non-opsonized particles.

Several groups have expanded functional studies of the lectin-dependent complement cascade to protochordates (T Fujita and M Nonaka) and echinoderms (LC Smith), but, to date, no functional assays have been published from more basal metazoan animals. Here we demonstrate: the production of the SeC3 protein and identify some of the C3b chains by western blot analysis; the localization of C3 in the basal metazoan *Swiftia exserta*, by cryo-immunohistochemistry; and that opsonized zymosan particles are phogytosed more rapidly by phagocytic cells from *Swiftia exserta* and by RAW 237.1 cells (a mouse macrophage cell line).

EPIGENETIC MODULATION INHIBITS T- REGULATORY CELL MEDIATED CD8$^+$ T CELL DYSFUNCTION IN LENTIVIRAL INFECTION

Mukta Nag, Yan Wang, Joanne Tuohy and Jonathan Fogle

Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, U.S.A.

For most HIV-infected individuals, virus-specific CD8$^+$ T cell activation is followed by dysfunction, which is characterized by poor T cell proliferation and decreased production of essential cytokines such as Interleukin-2 (IL2), Interferon γ (IFNγ) and Tumor Necrosis Factor α (TNFα). CD8$^+$ T cell activation can be characterized by an "activation phenotype" wherein there is a loss of CD62L and up-regulation of integrins such as CD11a, CD49d and CD11b. Activation of virus-specific CD8$^+$ T cells leads to changes in epigenetic conformation such as DNA demethylation and histone acetylation at the promoters of antiviral cytokine genes which are essential for their production. Using the feline immunodeficiency virus (FIV) model for lentiviral persistence, we have reported that immunosuppressive T regulatory (Treg) cells are activated early and progressively during the infection and decrease CD8$^+$ T cell IL2 mRNA and protein production (2). Using *ex-vivo* cell culture, we have demonstrated that Treg cells induce expression of the repressive transcription factor, Forkhead Box P3 (Foxp3) mRNA in virus-specific CD8$^+$ T cells at 1, 4 and 8 weeks post infection. *Based upon these findings, we hypothesize that epigenetic changes at the IL2 promoter, while essential for its production, make CD8$^+$ T cells highly accessible to Treg-induced, Foxp3-mediated suppression; and that blocking Foxp3 access will prevent suppression of IL2 production.* Using *in-vitro* cell cultures we have clearly identified a potent epigenetic modulator that can block DNA demethylation and histone acetylation in feline lymphocytes. Our data demonstrates that feline lymphocytes when modulated to retain their methylation and deacetylation, result in reduced Foxp3 binding at the IL2 promoter and show increased IL2 and Foxp3 mRNA levels compared to controls. Further, our *ex-vivo* results in virus-specific CD8$^+$ T cells confirm our hypothesis by demonstrating a reduction in Foxp3 enrichment at the IL2 promoter upon blocking demethylation and histone acetylation after autologous Treg co-culture. Collectively, these findings identify a novel mechanism that can open new avenues of therapeutic investigation to rescue virus specific CD8$^+$ T cell function for upcoming HIV cure and vaccination strategies.

CCTA-1 AND CCTA-2 ARE CANINE CANCER/TESTIS ANTIGENS

Paige Nemec, Jennifer Holmes, Alex Kapatos, Paul Hess

Department of Clinical Sciences, North Carolina State University, Raleigh NC

In solid tumors such as melanoma, only adoptive cell transfer (ACT) of *ex vivo*-expanded, autologous T cells has provided durable, complete responses in chemoresistant, advanced-stage patients. Targeting shared tumor-associated antigens (TAAs) offers the possibility of "off-the-shelf" ACT, but administration of T cells recognizing public differentiation TAAs have caused serious or fatal autoimmunity. Cancer-testis antigens (CTAs) are expressed during fetal development and in various cancers, but in few adult tissues, except for testis, an immunologically-privileged site. Tolerance and autoimmune risks are low, while sharing can be high, an ideal TAA profile. Using mass spectrometry to probe a canine histiocytic cell line, we found peptide signatures of 4 established human CTAs, and 12 other proteins whose human orthologs appear to be tissue-restricted (testis; testis/brain), according to multiple expression databases. We hypothesized that some may be novel, genuine canine CTAs in histiocytic sarcoma (HS), and evaluated mRNA expression of the 5 most-promising candidates (CCTAs 1-5) by qPCR in HS biopsies and a variety of normal tissues. In 10 HS samples, 9, 10 and 7 expressed CCTA-1, CCTA-2 and CCTA-3, respectively. CCTA-1 was highly expressed in two privileged sites (testis and brain), minimally expressed in the pituitary and marrow, and not detected in most somatic tissues or in the tumor parent, DCs. CCTA-2 was similar, but with higher expression in bone marrow. Other candidates had unfavorable peripheral expression (CCTA-3, CCTA-4) or were not found in HS samples (CCTA-5). CCTA-1 and CCTA-2 represent classical CTAs in the dog and can be developed as ACT targets.

CHARACTERIZATION OF THE MOST EVOLUTIONARILY CONSERVED NATURAL KILLER RECEPTOR, NKp30, IN EARLY VERTEBRATES

Yuko Ohta, Martin F. Flajnik

Department of Microbiology and Immunology, University of Maryland, Baltimore, Maryland

Natural killer receptors (NKR) are among the most rapidly evolving immune-recognition molecules. It is assumed that NKR continually undergo modifications to combat ever-changing viruses, and thus orthologous receptors are rarely detected among different vertebrate classes. In stark contrast to this paradigm, we identified *NKp30* genes from amphibians and sharks (human *NKp30* was found previously), revealing that NKp30 is the most conserved and oldest NKR. The *NKp30* gene in all vertebrates has a unique variable V-type domain resembling the precursor of antigen receptors (AgR), and thus likely predates the emergence of AgR. Sharks are the oldest living jawed vertebrates possessing an immune system similar to mammals, and therefore provide an excellent comparative model. In the nurse shark bacterial artificial chromosomes (BAC) clones spanning 300kbp, we identified nine *NKp30* genes with at least 5 different subtypes containing divergent V-type. Our preliminary results suggest that *NKp30* is linked to the major histocompatibility complex (MHC) in sharks. These observations support the hypothesis that AgR/NKR and MHC genes were syntenic early in vertebrate evolution. Moreover, shark NK cells were previously identified, however they were not characterized at the molecular level. In order to characterize NKp30-expressing NK cells, we generated polyclonal antisera against the highest expressed shark NKp30, and found that the NKp30 is also expressed by shark T cells. As NK and T cells are crucial for cellular immunity and are likely derived from a common “killer cell” precursor, NKp30 might be a common marker for both cell types in early vertebrates.

SOMATIC DIVERSIFICATION OF THE *SpTransformer* GENES; A SEA URCHIN IMMUNE GENE FAMILY

Matan Oren[1], Benyamin Rosental[2], Teresa Hawley[3], Gi-Young Kim[4], Jacob Agronin[1], Caroline Reinolds[1], Megan A. Barela Hudgell[1], L. Courtney Smith[1]

[1]*Department of Biological Sciences, The George Washington University*
[2]*Institute of Stem Cell Biology and Regenerative Medicine, Stanford University*
[3]*National Cancer Institute, NIH*
[4]*Department of Marine Life Sciences, Jeju National University*

Vertebrates depend on adaptive immunity for combating pathogens. A key feature of this system is the ability to diversify certain immune receptor genes (i.e. TCRs, Igs and VLRs) by recombination or copy choice plus hypermutation processes that take place in the soma of the organism. To date, there is very little evidence for similar somatic immune gene diversification in invertebrates. We report that a key immune effector gene family of the purple sea urchin, the *SpTransformer* (formerly *Sp185/333*) gene family, diversifies somatically in single immune cells of the same genotype by means of gene deletion, duplication and single point mutations. Gene amplicons in single cells can be different within individual animals relative to amplicons in sperm cells. We also show that all sequences of gene amplicons derived from single cells have full length open reading frames suggesting that the somatic diversification of the family does not generate pseudogenes and therefore may be regulated. Based on the genomic characterization of the family in single cells, two possible mechanisms may be involved in the gene diversification process. First, there may be a mechanism that is based on the positions of the short tandem repeats (microsatellites) within the gene clusters, and/or second, there may be a vertebrate-like recombination mechanism based on the expression of the sea urchin RAG-like genes.

SOMATIC HYPERMUTATION OF TCRα CONTRIBUTES TO THYMIC POSITIVE SELECTION IN SHARKS

Jeannine A. Ott[1], Caitlin D. Castro[2], Thaddeus C. Deiss[1], Yuko Ohta[2], Martin F. Flajnik[2] and Michael F. Criscitiello[1]

[1]*Comparative Immunogenetics Laboratory, Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843 USA*
[2]*Department of Microbiology and Immunology, School of Medicine, University of Maryland at Baltimore, Baltimore, MD 21201 USA*

In mammals and probably all vertebrates, receptor editing of TCR alpha genes enhances immature T cell positive selection over a three-day interval in the thymic cortex. Surprisingly we found extensive somatic hypermutation (SHM) operating at the TCRα locus in the nurse shark thymus, implying that SHM contributes to receptor modifications that enhance positive selection. We analyzed mutation in TCRα families of clones with the same VJ rearrangement. Additionally, *in situ* hybridization showed the strongest activation-induced cytidine deaminase (AID) expression in the central thymic cortex and bordering the corticomedullary junction, with weaker expression in the medulla. The frequency of mutation at TCRα was as high as that seen at B cell receptor (BCR) loci in sharks and mammals. Complementarity determining regions (CDRs) accumulated significantly more mutations than framework regions (FWs), and significantly more of CDR mutations resulted in amino acid replacement. We saw a preference for transition mutations as well as a strong bias toward G:C substitutions within AID hotspots, especially within CDR regions. We suggest that TCRα utilizes SHM to boost positive selection and perhaps to broaden diversification of the αβ T cell repertoire in sharks, the first reported use of this process in thymic diversification in vertebrates.

“MARCO” “POLO”: HOW RAINBOW TROUT CELLS FIND BACTERIA USING A CLASS A SCAVENGER RECEPTOR

Sarah J Poynter[1], Andrea Monjo[2], Gabriella Micheli[3], Stephanie J DeWitte-Orr[2,3]

[1]*Department of Biology, University of Waterloo, 200 University Ave W, Waterloo, ON*
[2]*Department of Biology, Wilfrid Laurier University, 75 University Ave W, Waterloo, ON*
[3]*Department of Health Sciences, Wilfrid Laurier University, 75 University Ave W, Waterloo, ON*

Class A scavenger receptors (SR-As) are a family of surface-expressed receptors who bind a wide range of polyanionic ligands including bacterial components and nucleic acids. These receptors play a role in a variety of cellular functions, including innate immunity. Macrophage receptor with collagenous structure (MARCO) is a SR-A family member that has been studied in mammals largely for its role in binding bacteria. To date there is little information about MARCO specifically and SR-As in general in fish, and which SR-As bind to bacteria remains largely unknown. In the present study two novel rainbow trout (rt)MARCO transcript variants have been identified; the deduced amino acid sequences have been analyzed for conserved domains and similarity to existing sequences. When overexpressed in CHSE-214, a Chinook salmon cell line that lacks functional scavenger receptors, GFP-tagged rainbow trout rtMARCO-1 and rtMARCO-2 were able to bind gram-positive, and gram-negative bacteria of both mammalian and aquatic sources. In mammals the scavenger receptor cysteine-rich (SRCR) domain is necessary for binding bacterial ligands, this was also in the case in rainbow trout, when the rtMARCO proteins were mutated to remove the SRCR the bacteria binding capability of both variants was lost. rtMARCO-1 and rtMARCO-2 did not show any binding to the yeast cell wall component zymosan or to double-stranded (ds)RNA. This is the first time rainbow trout MARCO sequences have been identified and the first in-depth study exploring their ligand binding profile. This study provides novel insight into the role of rainbow trout MARCO in innate immunity.

THE PROTEIN PROHIBITIN DISPLAYS SYSTEMIC ANTI-INFLAMMATORY AND ORGAN-PROTECTIVE EFFECTS DURING SEPSIS

Christine Psaltis[1], Brita Kilburg-Basnyat[1], Taylor Mattox[1], Kathleen Thayne[1], Jim Aloor[1], Jacques Robidoux[1], Ethan Anderson[2*], Kymberly Gowdy[1*]

[1]*Department of Pharmacology and Toxicology, East Carolina University, Greenville, NC*
[2]*Department of Pharmaceutical Sciences and Experimental Therapeutics, University of Iowa, Iowa City, Iowa*
[*]Authors contributed equally

Background: Sepsis, a systemic inflammatory response to infection, is a leading cause of mortality worldwide. Maintaining organ function during sepsis is critical to improve patient outcomes. Prohibitin (PHB), a ubiquitous protein, has multiple roles in mitochondrial structure, metabolism, and cell death. However, PHB's role in sepsis has been unexplored.

Methods: Using *in vivo* sepsis model, we injected C57Bl/6J mice i.p. with lipopolysaccharide (LPS). Following LPS, mice were given recombinant prohibitin (rPHB) i.p. Organs were analyzed for injury and inflammation. Cardiac function was measured via echocardiogram. Blood was used for systemic immune cell characterization by flow cytometry. HL1 cardiomyocytes overexpressing PHB exposed to TNF-α/IL-1β were used to measure mitochondrial function and pro-inflammatory cytokines.

Results: Systemic LPS decreased cardiac function but increased blood neutrophils (PMNs) ($CD45^+Ly6G^+$) and PMN expression of CD11b. However, rPHB restored LPS decreases in cardiac contractility and decreased CD11b expression. TNF-α/IL-1β increased pro-inflammatory cytokine expression and decreased mitochondrial function. However, when cardiomyocytes over-expressed PHB were incubated with TNF-α/IL-1β, mitochondrial dysfunction and increased pro-inflammatory cytokine expression were mitigated.

Conclusions: We found that PHB decreases systemic inflammation, decreases neutrophil maturation/recruitment and restores cardiac contractility during sepsis. PHB significantly improves mitochondrial function in cardiomyocytes. These findings illustrate PHB's diverse roles in enhancing mitochondrial function and regulating innate immunity.

UNRAVELING THE IMMUNOGLOBULIN SUPERFAMILY GENES GENOMIC ORGANIZATION IN CATFISH (*ICTALURUS PUNCTATUS*) BY THIRD GENERATION SEQUENCING

Sylvie M.A. Quiniou[1], Eva Bengtèn[2], Christophe Klopp[3], Bryan M. Musungu[1] and Mekki Boussaha[4]

[1]*Warmwater Aquaculture Research Unit, USDA-ARS-Stoneville, MS*
[2]*Department of Microbiology, University of Mississippi Medical Center, MS*
[3]*Genotoul, INRA, France*
[4]*UMR 1313 Génétique Animale et Biologie Intégrative, INRA, France*

The Immunoglobulin Superfamily (IgSF) gene regions have been amongst the most challenging ones to sequence and assemble due to the presence of multiple duplications and repetitive regions. The advent of third generation sequencing has enabled resolving most of these issues. We undertook sequencing of the major IgSF gene loci in catfish using Pacific Biosciences long read sequencing. First, we sequenced the genome of a meotic gynogenetic fish (CCBL1) for which we already had a BAC library and a physical map. Second, to ensure accuracy and contiguity of the large IgSF gene loci, we also sequenced 180 BACs, in parallel. The de novo genomic assembly produced a map of 1319 contigs with a N50 of 4.4Mb and a L50 of 50. Currently approximately 92% of the genome is anchored by a high density linkage map. Reintegration of the genome with the physical map and its 43,000 associated BES should further increase the anchoring by several percentages. Through BAC sequencing we assembled the regions encoding the antigen receptors of adaptive immunity: Immunoglobulin Heavy chains, the σ, κ and λ Immunoglobulin Light chains, the α, β, δ, and γ T cell receptors, as well as the major histocompatibility I and II. In addition, we also assembled the loci encoding teleost specific innate immune regulatory receptor families such as the LITRs, NITRs, PIGRLs and NILTs. The genome assembly results and the structural organization of IgSF gene regions will be presented.

GENE REGULATORY NETWORKS AND THE EVOLUTION OF IMMUNE CELL DEVELOPMENT

Jonathan P. Rast[1,2], Katherine M. Buckley[3], Catherine Schrankel[2], Nicholas Schuh[1], and Eric Ho[1,4]

[1]Department of Medical Biophysics; [2] Department of Immunology, University of Toronto, Toronto, ON
[3]Department of Biological Sciences, The George Washington University, Washington, D.C.
[4]National Cancer Institute, National Institutes of Health, Bethesda, MD

Cells that are specialized for immune defense are widespread through bilaterian animals but it is difficult to determine where evolutionary homology lies from a purely morphological perspective. Clearly similar cell types tend to be restricted within phyla and little detail is known about the molecular characteristics of immune cells in most animal groups. In principle developmental gene regulatory networks (GNRs) offer a level of comparison that can be used to solve this problem. We have characterized transcription factor function in the development of immunocytes in the purple sea urchin larva and have characterized regulatory interconnections that direct development from mesoderm to differentiated cell types. Similarities with vertebrates that suggest that regulatory circuitry is shared between chordates and echinoderms. As this type of information becomes more complete, hypotheses about the evolution of these cell systems will become more accessible to experimental validation and the utility of invertebrate models to shed light on more complex vertebrate systems will be increased.

VISUALIZING HOST MACROPHAGES-MICROBIAL PATHOGENS INTERACTIONS IN THE AMPHIBIAN *XENOPUS*

Jacques Robert and Kun Hyoe Rhoo

Department of Immunology & Microbiology, University of Rochester Medical Center, Rochester, NY

Cells of the monocytic lineage play a central role in host defenses, development and homoeostasis. However, the heterogeneity and plasticity of macrophages, depending on their developmental stage, tissue residence and types of activation, challenge the functional characterization of these cells. One way to gather more direct insights into their immunological role is to develop intravital animal models to visualize macrophage activity in real time. Using transgenesis to express fluorescent reporter genes under the control of cell type-specific promoters, we have characterized several transgenic lines, which label different subsets of myeloid lineages in tadpoles. These transgenic lines include *xlurp*:GFP (myeloid cells), *xmpeg*:GFP (mononuclear phagocytes), and double *xlurp*:GFP/*xmpeg*:mCherry (green myeloid cells such as granulocytes and yellow to orange macrophages). Beside to assessing the involvement of myeloid cells during infection with ranavirus FV3, we are currently adapting these transgenic lines for characterizing the role of macrophages in host immune defenses against *Mycobacteria marinum* (*Mm*). Notably, in combination with fluorescently labeled recombinant M*m*, we have been able to visualize M*m*-infected macrophages intravitally at the site of infection in the muscle tail, as well as to follow *Mm* dissemination to other organs such as liver and lung. We anticipate that our system will provide novel insights into the complex interactions of mycobacterial pathogens and host macrophages.

TECHNOLOGY TO DETERMINE HEALTH STATUS OF PRODUCTION ANIMALS IN A POINT-OF-CARE SETTING

M. E. Hockett[1], E. J. Jensen[1], B. A. Pike[1], J. Pollard[1], A. M. Saxton[2], R. R. Rodriguez[1,3]

[1]*Advanced Animal Diagnostics, Inc., Morrisville, NC, 27560,*
[2]*Institute of Agriculture, University of Tennessee, Knoxville, TN, 37996*
[3]*North Carolina State University College of Veterinary Medicine, Raleigh, NC, 27601*

Animal health status and well-being is often most noticed when animals are clinically ill and there are visible symptoms of a health event. However, technology that has previously been available in a point-of-care setting for human medicine is now available in the animal health industry and allows for animal-side blood leukocyte differentials (QScout® BLD). Query of the immune system to determine health status may have value to understand when an individual animal or pens/groups of animals may be facing an immunological challenge. Furthermore, such technologies may have value to help provide objective data and information to guide judicious use of antibiotics. Antibiotic usage in livestock production is under intense scrutiny due to concerns about the development of antibiotic resistant organisms. However, antibiotics play an important role in the health and welfare of infected or at-risk animals, especially at times of stress and comingling. A recent feedlot trial examined if a) results of the blood leukocyte differential were predictive of animals with poor performance and b) if treatment of cattle identified by blood leukocyte differental with an anti-microbial could prevent impaired performance. Moderate-risk cattle (n=1,554; 57.7% heifers, 42.3% steers) weighing 650 (±300) pounds were randomly assigned to receive gamithromycin or no treatment and were tested using QScout BLD upon arrival to a feedyard. High and low thresholds were determined for neutrophils and lymphocytes that were predictive of average daily gain and morbidity events. The blood leukocyte differential results were used to retrospectively assign calves to a “normal” (NRM) or “abnormal” (ABNRM) BLD status. Using the high and low thresholds for neutrophils and lymphocytes that were developed, 13.2% of animals were found to have ABNRM statuses. Calves designated by BLD as ABNRM had reduced gain with great morbidity, but treatment with gamithromycin prevented these losses. However, there was no improvement in gain when treatment was provided to NRM calves. Restricting arrival treatment to calves with QScout BLD results outside the normal range would have resulted in 86.8% reduction in arrival antibiotic use compared to metaphylaxis. This study demonstrated that testing calves upon arrival to the feedyard with QScout® BLD identified a group of animals that benefited from treatment by reduced morbidity and increased weight gain to harvest. The use of QScout BLD shows promise as a tool to monitor animal health and promote animal welfare by providing objective data to guide management and treatment decisions for production animals (such as cattle) while also supporting judicious and warranted use of anti-microbials.

THE ROLE OF TMEM150A AS A NOVEL REGULATOR OF CYTOKINE SIGNALING

Jessica Romanet, Katherine Cupo, Gretchen Scheffe, Jeffrey A. Yoder

Department of Molecular Biomedical Sciences, North Carolina State College of Veterinary Medicine, Raleigh, NC, USA

A healthy immune response must include an inflammatory cascade to recognize, defend against, destroy and remove an insulting source, while mitigating the damaging effects against the host's tissues from a prolonged and exacerbated immune response. In this process, cytokines, which can be released by numerous cell types, play an important role in recruiting inflammatory cells to sites of infection or injury. Understanding the factors that regulate cytokine release is not only critical to understanding the cellular biology of the immune response, but is also vital for identifying new therapeutic targets for controlling unchecked cytokine production in a wide range of diseases. Unchecked cytokine levels contribute to a wide range of illnesses, including septic shock, cancer growth and metastasis, asthma, reperfusion injury, psoriasis, periodontal disease, and inflammatory bowel disorders. In order to better understand these processes, we have identified *TMEM150A*, a novel, highly conserved and heretofore functionally undefined gene, as a regulator of cytokine production. Knock-down of *TMEM150A* expression in epithelial cells resulted in significantly altered levels of cytokine transcripts and secreted cytokine proteins. These observations suggest that TMEM150A plays an important role in regulating cytokine response, and, that it, and the pathway it affects, may provide novel targets for controlling common inflammatory diseases.

INTERACTIONS BETWEEN MICROBIOTA AND THE TELEOST IMMUNE SYSTEM IN HEALTH AND DISEASE

Irene Salinas

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

The microbial communities that live at the mucosal surfaces of all animals create one of the most ancient and successful symbiotic partnerships found in nature. Shifts in the environment or the host as well as pathogen infection alter the delicate balance between microbiota and host mucosal surfaces. Recent deep-sequencing surveys have untapped the diversity of the bacterial microbiota of a number of fish species. However, how symbiont populations are maintained by the fish host is still poorly understood. Here. I will summarize the known mechanisms by which symbionts are recognized by the rainbow trout immune system under homeostatic conditions. I will present our latest results regarding the interactions between fish symbiotic bacteria and the mucosal immune system, with a focus on the trout secretory component. I will also give examples on how symbiont metabolites such as sphingolipids directly orchestrate mucosal antibody and B cell responses. Finally, I will give some examples on how the Atlantic salmon host-microbiota interactions are perturbed in the skin during the course of a viral infection. Our results take us a step closer to understanding the complex co-evolutionary processes that shape the partnership between microbiota and vertebrate mucosal surfaces.

BROADENING THE SCOPE OF GASTROPOD IMMUNITY: INSIGHTS FROM A NEW SNAIL HOST/PARASITE MODEL

Jonathan H. Schultz, Coen M. Adema

Center for Evolutionary and Theoretical Immunology, Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA

Current insights of gastropod internal defense systems are largely based on a few model organisms that are important for disease transmission or represent economic food sources. Such a narrow scope may not be representative of immune function for a diverse class like the Gastropoda (~60,000 species). The transcriptome and genome of *Physella acuta*, an understudied gastropod in terms of immunity, was captured using next-generation sequencing (454, Illumina). This also yielded FREP sequences: immune factors known to be somatically mutated and involved in the anti-trematode defense response of *Biomphalaria glabrata*, a planorbid snail within a sister family of *P. acuta*. We recorded ~5 FREP sequences, a dramatic reduction in gene sequence number compared to *B. glabrata* (> 20 sequences). Furthermore, sub-cloning and sequencing of a partial amplicon of FREP1 in *P. acuta* did not yield variant sequences suggesting that this FREP is not somatically mutated. To determine the role of FREPs in anti-trematode defense we exposed *P. acuta* snails to the parasite *Echinostoma paraensei* and analyzed protein composition (SDS-PAGE) of *P. acuta* plasma at 0,2,4, and 8 days post exposure (DPE). FREPs are not prominently part of anti-parasite responses. Additionally, we obtained Illumina RNA-Seq data to analyze FREP gene expression at 0,2, and 8 DPE. In conclusion, study of a novel snail host/parasite model supports the notion that *P. acuta* FREP genes are reduced in number and lack somatic diversification. Also, immune function differs between the Planorbid and Physid snail families.

DIVERSIFICATION OF CHEMOKINE CCL19 GENES IN SALMONIDS AND THEIR ROLE IN RAINBOW TROUT NASAL IMMUNITY

Ali Sepahi, Elisa Casadei, Irene Salinas

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

Chemokines are small cytokines with several immune roles including migration and compartmentalization of lymphocytes. Chemokine genes have diversified in teleosts but the functional consequences of this diversification are poorly understood. Previously, chemokine CCL19 was found to be the main innate immune gene up-regulated in the nasal tissue of rainbow trout following intranasal vaccination with a viral vaccine. The goal of this study was to characterize all the CCL19 genes in salmonids and identify which of these genes have specialized roles in nasal immunity. We performed bioinformatics data searches using previously published CCL19 sequences for teleosts and other vertebrates. Results show a dramatic expansion of CCL19 genes in salmonids, with 7 genes identified in the trout genome. These genes share very low sequence identities and are located in four different chromosomes in Atlantic salmon. Tissue distribution studies and in vivo vaccination studies show that CCL19a (-1 and -2) is the main CCL19 form expressed in mucosal lymphoid tissues and responsive to nasal viral vaccination. In vivo nasal delivery of trout recombinant CCL19a1 induced enlargement of the nasal lamina propria, morphological changes in nasal MHC-II$^+$ cells, increase in numbers of nasal CD8$^+$ cells and up-regulation of immune genes related to antigen presentation and antiviral cellular immune responses. Our results demonstrate that expansion of CCL19 genes in salmonids resulted in acquisition of molecules with specialized mucosal immune roles, in this case nasal antiviral immune responses.

EVOLUTION OF THREE TANDEM COPIES OF THE INTERLEUKIN-1 RECEPTOR-LIKE 1 GENE IN SALMONID FISH AND THEIR CONTRIBUTION TO RAINBOW TROUT BACTERIAL COLD WATER DISEASE RESISTANT PHENOTYPE

Cassidy H. Shaw, Sixin Liu, Guangtu Gao, Yniv Palti, and Gregory D. Wiens

National Center for Cool and Cold Water Aquaculture, Agricultural Research Service, United States Department of Agriculture, 11861 Leetown Road, Kearneysville, WV 25430, USA

Rainbow trout exhibit extensive phenotypic variation in innate disease resistance. Five generations of family based selection has resulted in rainbow trout lines with either increased or reduced survival following exposure to the gram-negative bacterium, *Flavobacterium psychrophilum (Fp)*, the causative agent of bacterial cold water disease (BCWD). Whole body RNA-seq analysis and RT-PCR of spleen tissue samples identified lower basal level expression of interleukin-1 receptor-like 1 (*il1rl1*) in susceptible line fish as well as decreased expression in response to infection. Genomic analyses identified three tandem, *il1rl1* genes on chromosome 3 located in a previously identified, BCWD QTL region. We report the genomic organization and evolution of three *il1rl1* genes, and develop gene specific assays to measure the expression of individual *il1rl1* genes in susceptible and resistant rainbow trout lines. Sequence homology between these three putative genes is approximately 95%. Each gene contains a signal peptide, three IG/IG-like domains, a transmembrane region and a TIR domain. Two tandem *il1rl1* genes and one pseudogene are present in Atlantic salmon (*Salmo salar*) and Coho salmon (*Oncorhynchus kisutch*) genomes with 86-94% sequence identity to rainbow trout *il1rl1* genes. Low homology (20-25%) was observed between trout *il1rl1* genes and those of higher vertebrates. Phylogenetic analyses grouped trout *il1rl1* with *il1rl1* sequences from other species and apart from other genes of the interleukin 1 receptor family and suggest that the salmonid *il1rl1* genes expanded by an ancestral tandem duplication in the salmonid linage. Differential expression analyses will be used to determine the contribution of *il1rl1* gene copy number to the disease resistant phenotype.

AN ESSENTIAL ROLE OF IgT IN HOST-MICROBIOAL HOMEOSTASIS

Fumio Takizawa[1], Zhen Xu[2] Yasuhiro Shibasaki[1], Thomas Sauters[3], Irene Salinas[3], J. Oriol Sunyer[1]

[1]*University of Pennsylvania, Philadelphia, PA, USA*
[2]*Department of Aquatic Animal Medicine, Huazhong Agricultural University, Wuhan, China*
[3]*Department of Biology, University of New Mexico, Albuquerque, NM, USA*

The vast majority of fish pathogens enter their host through mucosal surfaces. We have demonstrated in rainbow trout that pathogen-specific mucosal antibody responses are mediated IgT. In addition, we have shown that IgT is the main immunoglobulin isotype coating commensal bacteria. While these IgT activities point to a pivotal role of this immunoglobulin in teleost mucosal immunity, whether IgT is required for pathogen clearance and microbiota homeostasis remains to be demonstrated. Here we studied whether IgT is required for microbiota homeostasis. To address this question, we developed an IgT^+ B-cell depletion model in rainbow trout. Upon one depletion treatment, IgT^+ B cells were depleted by over 95% in all tested mucosal and systemic lymphoid organs. Strikingly, these cells remained depleted for a 3-4 week period. After two weeks of the treatment, the percentage of microbiota coated by IgT was drastically reduced from ~40-50% to ~0-2%. In addition, we found a high amount of microbiota translocated into the gill tissue of IgT-depleted fish. This translocation correlated with the upregulation of key pro-inflammatory cytokines as well as with substantial alterations in gill morphology. Critically, significant changes were detected in the gill microbiome of IgT-depleted fish, thus confirming further the induction of dysbiosis in the gill as result of IgT depletion.

In conclusion, we demonstrate that IgT plays a critical role in the maintenance of microbiota homeostasis in a fish mucosal surface. We anticipate this novel IgT^+ B-cell depletion model will be important to understand further the role of IgT in host-microbiota interactions.

IMMUNE FUNCTION IN SMALLMOUTH BASS (*MICROPTERUS DOLOMIEU)* FROM THE CHESAPEAKE BAY WATERSHED

Cheyenne Smith[1,2], Chris Ottinger[1], Vicki Blazer[1], and Luke Iwanowicz[1]

[1]*U.S. Geological Survey, National Fish Health Research Laboratory, Kearneysville, WV*
[2]*Department of Environmental Biology, Hood College Graduate School, Frederick, MD*

The Potomac and Susquehanna Rivers, both tributaries to the Chesapeake Bay, are sites that have experienced major fish kills beginning in 2002 for adults (Potomac) and 2005 for young of the year (Susquehanna). Fish health investigations indicated smallmouth bass from both river basins have a variety of bacterial infections, heavy parasite loads, and sometimes viral and fungal infections. Because no single or consistent cause can be identified, we believe the fish are immunosuppressed due to contaminants and other stressors and thus susceptible to many opportunistic pathogens. Fish were collected from four sites with varying levels of agricultural land use and wastewater input. Anterior kidney tissue was removed from 20 adult smallmouth bass at each site. Samples were collected across three sampling seasons beginning in spring 2016 and continuing to spring 2017. Bactericidal killing ability, respiratory burst activity, and lymphocyte mitogenesis were used as measures of immune function. We modified classical diagnostics for these assays, which involves spectrophotometric analysis, to image-based flow cytometry. This allowed collection of single cell reads and determination of responses for individual cells classified based on width, cell surface markers and nuclear morphology. For mitogenesis, the main differences found were between our reference site (high forested, low agriculture) and high agricultural site for cells that were 10 μm and 12 μm in width. Oxidative stress appeared to play a role in these differences. Additional analyses are still underway and the immune function results are being integrated with water contaminant concentrations measured monthly, tissue histopathology and plasma analyses.

CONSEQUENCES OF PRENATAL ANDROGEN EXPOSURE FOR OFFSPRING HEALTH: AN EXPERIMENTAL STUDY IN WILD MEERKATS

Kendra Smyth[1,2], Alexandra Stonehill[3], Nick Caruso[4], Christine Drea[3]

[1]*University Program in Ecology, Duke University, Durham, NC*
[2]*The Kalahari Research Trust, Kuruman River Reserve, South Africa*
[3]*Department of Evolutionary Anthropology, Duke University, Durham, NC*
[4]*University of Alabama, Tuscaloosa, AL*

Androgens underlie a well-known tradeoff between reproductive benefits versus health costs in males. Despite substantial variation in female androgen production and the potential for transgenerational effects, this tradeoff is underappreciated in females and their offspring. In the cooperatively breeding meerkat (*Suricata suricatta*), all females are hormonally 'masculinized,' but the dominant female within each clan has greater androgen concentrations than does any subordinate female (Davies et al. 2016). Her raised androgen concentrations presumably increase her competitive abilities but are also associated with immunosuppressive costs (Smyth and Drea 2016; Smyth et al. 2016). Here, we ask if exposure to raised prenatal androgens produces a comparable trade-off in meerkat offspring. From 2012-15, we measured parasite burdens and innate immune responses of offspring derived from dominant and subordinate control dams, and from dominant dams treated with an androgen-receptor blocker. For the first time in a wild mammal, we report that prenatal exposure to androgens has immunosuppressive effects. The offspring of dominant control dams, that purportedly had been exposed to greater androgen concentrations prenatally, experienced greater parasite burdens relative to the offspring of subordinate control dams. Although normative status-related differences in prenatal androgen concentrations were unrelated to offspring immune function, experimentally blocking the actions of prenatal androgens improved offspring immunocompetence. Thus, despite the reproductive benefits of hormonal masculinization for those female meerkats that emerge as dominant, the transgenerational consequences of raised maternal androgens for immune function are naturally experienced by all juvenile meerkats and appear to represent a cost of sexual selection operating in females.

POLDNAVIRUSES AS MUTUALISTS AND IMMUNOSUPPRESSIVE AGENTS OF INSECTS

Michael R. Strand

Department of Entomology, University of Georgia, Athens, GA

Insects rely upon a well-coordinated innate immune system for protection against foreign invaders. Pathogens and parasites have reciprocally evolved a diversity of counterstrategies for suppressing host insect defenses. Among the most important mortality agents of insects are thousands of parasitoid wasp species that carry polydnaviruses (PDVs). PDVs persist as proviruses in wasps and replicate asymptomatically in the reproductive tract of females. Wasps reproduce by laying eggs into hosts that progeny consume. During egg laying females also inject PDV particles into hosts. PDVs do not replicate in the hosts of wasps but expression of PDV-encoded genes causes physiological alterations that are essential for survival of wasp offspring. Thus, a mutualism exists between PDVs and wasps as viral transmission depends on parasitoid survival and parasitoid survival depends on infection of hosts by the virus. Genome analysis indicates that PDVs in the genus Bracovirus evolved from a group of viruses that are virulent insect pathogens. Novel alterations in genome organization and function underlie why bracoviruses persist and cause no disease in wasps, yet produce virulence gene products in the hosts of wasps. Functional studies indicate that several of these virulence genes target immune pathways, which disables defenses that otherwise would kill wasp offspring.

AN EVOLUTIONARILY ANCIENT INNATE IMMUNE ROLE IDENTIFIED IN PLASMA CELLS

Jacob Paiano[1], Fumio Takizawa[1], Yasuhiro Shibasaki[1], Joel Wilmore[2], David Allman[2], J. Oriol Sunyer[1]

[1]*Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, 19104*
[2]*Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104*

Antibodies are the crown jewel of humoral adaptive immune responses and, plasma cells (PCs) are by far the most specialized and main antibody-secreting cells. Until very recently, PCs were viewed exclusively as antibody-producing factories. However, this perception is starting to shift as immunomodulatory roles for PCs have recently been identified. In line with that changing scenario, here we show for the first time that terminally differentiated murine PCs efficiently perform phagocytosis, a function regarded as a crucial mechanism of innate immunity. More specifically, we show that a large subset of splenic PCs in mice have a strikingly high phagocytic capacity. We also show that phagocytic plasma cells (P_hPCs) could phagocytose *E. coli* and latex beads, and that by transmission electron microscopy, sorted P_hPCs have the typical morphology of a PC, which is characterized by abundant rough endoplasmatic reticulum (RER) in the cytoplasm. Critically, upon LPS injection into the mouse peritoneal cavity, we observed a ~16 fold increase in the number of P_hPCs, which represented ~1% of the total splenocytes. This dramatic increase in the number of P_hPCs three days after LPS injection strongly suggests a role for P_hPCs early in infection. This role appears to be evolutionarily conserved as similar cells were detected in rainbow trout, a teleost fish. Overall, here we provide the first demonstration that PCs, the most central component of adaptive immunity, also play a key evolutionarily ancient innate immune role, and thus, our discovery represents a paradigm shift in our understanding of PCs function.

ON THE EVOLUTIONARY ORIGINS OF CD1D AND SEMI-INVARIANT NATURAL KILLER T CELLS

N Suryadevara[1,*], A Kumar[1,*], P Chimski[1], K Oh[1], C Caster[2], Richard W Truman[3], Liming Ren[4], M Criscitiello[2], & S Joyce[1]

[1]*VA Tennessee Valley Healthcare System; Vanderbilt University Medical Centre, Nashville, TN*
[2]*Texas A&M University, College Station, TX*
[3]*School of Veterinary Medicine Louisiana State University Baton Rouge, LA*
[4]*State Key Laboratory of Agro-biotechnology, China Agricultural University, Beijing, China*
*Contributed equally to this work

The evolutionary origins of CD1d molecules and the T cell receptors (TCR) that recognize them currently remain unknown. CD1d molecules control the functions of a specialized subset of lymphocytes called semi-invariant natural killer T (type I NKT) cells. Type I NKT cells recognize both the self and non-self agonist —α-galactosylceramide (αGC) and related glycolipids— when presented by CD1d molecules. NKT cells express an invariant TCR α-chain (iTCRα) resulting from the mouse *Vα14* or human *Vα24* to *Jα18* rearrangement. iTCRα pairs with the mouse Vβ8 (Vβ7, Vβ6, Vβ2) or the orthologous human Vβ11 TCR β-chain to create a functional NKTCR. Our phylogenetic analyses revealed that whilst the mouse *Cd1* homologs evolved as far back as the anole lizards, the orthologues of *Cd1d* and that of *Vα14/Vα24* and *Jα18* gene segments emerged with the decent of mammals. Moreover, the known avian CD1 orthologs lack the α3 domain and both the reptilian and the avian CD1 lack a functional endo/lysosomal-recycling motif critical for NKT cell development and function. As well, monotreme genomes lack *Cd1d, Vα14/Vα24* and *Jα18* orthologs and the marsupial *Cd1d, Vα14/Vα24* and *Jα18* orthologs harbor indels that would render the encoded proteins non-functional. Consistent with these findings, one of the most divergent *Cd1d* gene isolated from the nine-banded armadillo *Dasypus novemcinctus* and ectopically expressed in a cell line presented αGC and activated mouse NKT cell hybridomas. Hence, we conclude that the CD1d-restricted αGC-presentation in the immune system is an eutherian innovation.

COMBINATORIAL VACCINE DESIGN USING MOTIF FINGERPRINTS TO COUNTER KNOWN AND UNKNOWN PATHOGENS

Willy Valdivia[1], Royston E. Carter[2]

[1]*Orion Integrate Biosciences Inc., 2005 Research Park Circle, Manhattan KS 66502*
[2]*Marine Bio-Technologies Center of Innovation (MBCOI), 5600 Marvin K. Moss Lane, Wilmington, NC 28409*

Here we summarize the development of an advanced computational and synthetic biology approach to rapidly design and express broad spectrum combinatorial vaccines capable to elicit broad protective immune responses against several pathogen species of biodefense relevance. Our approach combines advanced genomic-based computational analysis algorithms to discover and prioritize lead-candidate antigens. These epitopes are prioritized for their binding affinity to different alleles of the HLA-I or/and HLA-II. Recombinant proteins that have been generated by rational and combinatorial protein engineering strategies are used in subunit vaccine designs using lambda bacteriophages. The number and combination of motif fingerprints required to elicit a ***spectrum combinatorial vaccine*** is synthetized conjugated. At the core of our effort lays a new genome analysis algorithms and database management systems that scan a pathogen genome and identify segments that are conserved and specific. This target selection and prioritization process has identified protein segments conserved and specific across several species of viruses and bacteria. These ***motif fingerprints (MF)*** do not overlap, but they may be contiguous in 3-dimensional protein space. Our effort will significantly reduce the time needed to start vaccine output and exploit next generation sequencing to develop prophylactic targets for newly discovered pathogens. When in the complex structure of a protein. This project will provide important insights into the development of new generation biodefense vaccines and the development of a rapid vaccine platform to protect against known and unknown biothreats. combinations of inserts in the same or different vector platforms increase the breadth of coverage in vaccines.

UNRAVELLING CELLULAR PERMISSIBILITY OF NORTH AMERICAN TADPOLES TO FROG VIRUS 3 (FV3): AN INITIAL OBSERVATION WITH A NEW TADPOLE CELL CULTURE FROM THE AMERICAN TOAD (*Anaxyrus americanus*)

Nguyen T. K. Vo[1], Levi C. Moore[1], Joshua Everson[1], Eric Leis[2], Stephanie J. DeWitte-Orr[1,3]

[1]*Department of Health Sciences, Wilfrid Laurier University, Waterloo, ON, Canada*
[2]*La Crosse Fish Health Center, U.S. Fish and Wildlife Service, U.S. Department of the Interior, La Crosse, WI, USA*
[3]*Department of Biology, Wilfrid Laurier University, Waterloo, ON, Canada*

Permissibility to ranavirus infection is considered one contributor of the global amphibian population decline. To date, ranavirus infections have been documented in at least 72 amphibian species in 14 families on 5 continents; including *Bufonidae* to which the American Toad belongs. Frog virus 3 (FV3) is the type species for the ranavirus family. Interestingly, juvenile frogs such as tadpoles appear to be more susceptible to FV3 infection compared to adult life stages; likely due to differences in immune response capabilities between life stages. And while North American frogs have experienced mass mortalities due to FV3 infection, most studies with FV3 have been using the African clawed toad (*Xenopus laevis*). We have successfully developed a new continuous tadpole cell line from *A. americanus*. Primary cell cultures were established from a homogenized whole tadpole. The cell line, named as BufoTad, is an epithelial cell line with little senescence-associated β–galactosidase activity. BufoTad has been passaged 25 times in a reduced Leibovitz's L-15 medium with 10 % FBS and is negative for FV3 transcripts. BufoTad is susceptible to FV3 infection and supports FV3 replication at 17-25^{o}C. Temperature appears to have an effect on virus infectivity, with greater cytotoxic effects at high temperatures. Further cellular and molecular analyses are underway to further understand interactions between FV3 and BufoTad cells.

BHF ALELIC POLYMORPHISM DETERMINES CHIMERIC STATE STABILITY IN *Botryllus schlosseri*

Ayelet Voskoboynik[1,2] Mark Kowarsky[3], Benyamin Rosental[1,2], Aaron M. Newman[1], Kohji Hotta[4], Katherine J. Ishizuka[1,2], Karla J. Palmeri[1,2], Norma F. Neff[5], Stephen R. Quake[5,6] & Irving L. Weissman[1,7]

[1]*Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, USA*
[2]*Hopkins Marine Station, Stanford University, USA*
[3]*Department of Physics, Stanford University, USA*
[4]*Department of Biosciences and Informatics, Keio University, Japan*
[5]*Chan Zuckerberg Biohub, USA*
[6]*Departments of Applied Physics and Bioengineering, Stanford University, USA*
[7]*Ludwig Center for Cancer Stem Cell Research and Medicine, Stanford University, USA*

Histocompatibility is the basis by which multicellular organisms of the same species distinguish self from nonself. To gain insights into the evolution and molecular characteristics of allorecognition, we are studying *Botryllus schlosseri* a member of the tunicates. *B. schlosseri* that engages in a natural transplantation reaction, whereby self-recognition between colonies leads to the formation of chimeras with shared vasculature (fusion), and a non-self recognition results in rejection. Progeny from crosses between histocompatible *B. schlosseri* colonies are known to segregate as expected from a monogenic trait. We have found a polymorphic gene, called BHF, or *Botryllus* histocompatibility factor, that governs fusion or rejection outcomes between *Botryllus colonies*. Colonies fuse if they share one or both BHF alleles AB=AB or AB=BC and reject each other if there are no alleles in common, e.g. AB=CD (Voskoboynik and Newman et al. 2013). Following fusion, one chimeric partner is often eliminated in a process of allogeneic resorption (Corey and Rosental et al. 2016). But stable chimerism where both partners thrive also occurs. Here based on long term studies aims to characterize molecular and morphological phenotypes of chimeras we provide evidence that BHF, and genes that promote immune response and cell death, are highly expressed in the eliminated chimeric partner. Furthermore, the level of allelic differences between the non-shared BHF allele (e.g. A vs. C in AB=BC chimera) determines short-term/stable chimerism phenotypes. Just as HLA haplotyping predicts the success of organ transplantation in humans, BHF haplotyping can predict both fusion/rejection outcomes and, loss of tolerance in the *Botryllus* chimera.

GENOMIC CHARACTERIZATION OF NOVEL IMMUNOGLOBULIN-LIKE TRANSCRIPTS (NILTS) IN TELEOSTS

Dustin J. Wcisel[1], Sean C. McConnell[2], Jill L.O. de Jong[2], Gary W. Litman[3], and Jeffrey A. Yoder[1,4]

[1]*Functional Genomics Program, North Carolina State University, Raleigh, NC USA*
[2]*Department of Pediatrics, The University of Chicago, Chicago, IL USA*
[3]*Department of Pediatrics, University of South Florida Morsani College of Medicine, St. Petersburg, FL USA*
[4]*Department of Molecular Biomedical Sciences, North Carolina State University, Raleigh, NC USA*

The immunoglobulin (Ig) superfamily (IgSF) includes membrane bound and secreted proteins which possess one or more Ig domains that share core conserved residues and structural features with immunoglobulins (e.g. antibodies). Numerous IgSF members play significant roles in immune function and hundreds of IgSF members have been identified within genomes of various teleost fish species. For example, novel Ig-like transcripts (NILTs) that encode membrane bound proteins possessing one or two Ig domains have been identified in carp, salmon, and trout. Here, we investigate the NILT gene family in light of recent genome sequencing projects. We characterize NILT sequences in the zebrafish genome which encode a highly expanded and diverse cluster of NILTs compared to other teleost species. At least two gene content haplotypes are observed at a single locus on chromosome 1 within standard lab lines of zebrafish. Although numerous NILTs encode either activating or inhibitory signaling motifs, their cellular function remains undefined. Differential patterns of tissue expression and alternative mRNA splicing further expands the repertoire of NILTs. Utilizing spotted gar to bridge the gap between teleost and humans, we propose that NILTs may represent an alternative evolutionary pathway taken by the precursors of mammalian CD300/CMRF-35 and polymeric immunoglobulin receptor (pIgR) genes.

THE ROLE OF HIGH-DENSITY LIPOPROTEIN (HDL) IN PULMONARY IMMUNITY

MJ Yaeger[1], S Reece[2], B Kilburg-Basnyat[2], B Luo[2], MB Fessler[4], MJ Thomassen[3], KM Gowdy[2]

[1]*Department of Biomedical Engineering, East Carolina University, Greenville, NC*
[2]*Department of Pharmacology and Toxicology, East Carolina University, Greenville, NC*
[3]*Department of Internal Medicine, East Carolina University, Greenville, NC*
[4]*National Institute of Environmental Health Sciences, Research Triangle Park, NC*

Background: Recently, studies have reported an inverse correlation between levels of serum HDL and severity of lung diseases. However, how HDL communicates with the lung is still unknown. We hypothesize that HDL is critical in preventing pulmonary injury, in part, through its interaction with lipoprotein receptors during pulmonary inflammation. This will be tested utilizing exposure to aerosolized LPS, a standard model that induces pulmonary injury.

Methods: C57BL/6J mice were dosed intravenously through the retro-orbital sinus with either PBS or HDL before exposure to aerosolized LPS. Mice were necropsied 24hrs after exposure for lung tissue and BAL to determine BAL cell differentials, BAL cholesterol, and mRNA expression of lipoprotein receptors (SR-BI, ABCA-1, ABCG-1, LDLr, and CD36), pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) and chemokines (KC and MIP-2), and adhesion molecules (ICAM-1 and VCAM).

Results: LPS exposure significantly increased BAL neutrophils, BAL cholesterol, and pulmonary expression of ABCG1, ABCA1, SR-BI, LDLr, ICAM-1, and all pro-inflammatory cytokines/chemokines when compared to unexposed mice. HDL pretreatment significantly reduced LPS induced lung neutrophilia, pulmonary expression of KC, and IL-1β, and downregulated ABCG1, ABCA1, SR-BI, and LDLr when compared to control. Pulmonary expression of ICAM-1 was increased with LPS exposure, however ICAM-1 and VCAM were significantly suppressed with HDL pretreatment before LPS exposure.

Conclusions: Mice pretreated with HDL were found to have a decrease in pulmonary neutrophilia, select pro-inflammatory cytokines/chemokines, adhesion molecules, and lipoprotein receptor gene expression after LPS exposure. These data indicate HDL mitigates pulmonary immune cell influx, and influences lipoprotein receptor expression during lung inflammation.

DIFFERENTIATION-DEPENDENT ANTIVIRAL CAPACITIES OF AMPHIBIAN (*Xenopus laevis*) MACROPHAGES

Amulya Yaparla and Leon Grayfer

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

Colony stimulating factor-1 (CSF-1) is the principal macrophage (Mϕ) growth factor; indispensable to macrophage survival, proliferation and differentiation. CSF-1 binds to the CSF-1 receptor (CSF-1R), expressed on committed macrophage-lineage precursors and derivative populations. Recently, interleukine-34 (IL-34) has been identified as an alternate CSF-1R ligand and in the amphibian *Xenopus laevis* this cytokine gives rise to morphologically and functionally distinct Mϕs to those derived by CSF-1. Notably, while the *X. laevis* bone marrow-derived, CSF-1-differentiated Mϕs are highly susceptible to the emerging Frog Virus 3 (FV3) ranavirus, IL-34 derived Mϕs are resistant to this pathogen. Since antiviral interferon (IFN) cytokines are integral to vertebrate antiviral immunity, we examined the expression of these genes in CSF-1 and IL-34 Mϕs to account for their differences in antiviral capacities. IL-34 Mϕs showed robust gene expression of several antiviral IFN cytokines along with their respective receptors. By contrast, CSF-1 Mϕs exhibit modest IFN ligand and cognate receptor gene expression, presumably accounting for their less-effective antiviral capabilities. Cellular resistance to viral replication is controlled by a plethora of cellular mechanisms, collectively referred to as restriction factors. Interestingly, IL-34 Mϕs possessed significantly greater gene expression of select restriction factors than CSF-1 Mϕs. Finally, we demonstrated that IL-34 Mϕ-conditioned supernatants conferred anti-FV3 protection to the virally susceptible *X. laevis* kidney cell line (A6). Together, this work defines the mechanisms facilitating the cogent anti-FV3 capacities of IL-34 Mϕs in comparison to CSF-1 derived Mϕs.

EXAMINATION OF FISH IMMUNOREGULATORY RECEPTOR-MEDIATED SIGNALING EVENTS

Myron A. Zwozdesky, Chenjie Fei, Hima Gurupalli and James L. Stafford

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

Cells sense and respond to their environment through transmembrane receptors that transduce extracellular cues into biochemical signaling. While many mammalian receptor signal transduction events are well characterized, including Fcγ receptor (FcγR)-mediated phagocytosis, information from earlier vertebrate models is limited. The channel catfish (*Ictalurus punctatus*) leukocyte immune-type receptor (IpLITR) family consists of multiple receptor-types with variable signaling abilities that are dependent on their tyrosine-containing cytoplasmic tail (CYT) regions to control various innate immune cell effector responses. IpLITR 2.6b associates with the immunoreceptor tyrosine-based activation motif-containing adaptor molecule IpFcRγ-L, and when expressed in mammalian cells activates conserved effector responses, including phagocytosis similar to mammalian FcγRs. Conversely, IpLITR 1.1b is a long immunoreceptor tyrosine-based inhibitory motif-containing receptor with multi-functional capabilities. IpLITR 1.1b-mediated inhibition of cellular cytotoxicity is facilitated by a distal CYT region SHP1-dependent and a proximal CYT region Csk-dependent mechanism. Interestingly, IpLITR 1.1b also activates a unique F-actin-dependent phagocytic pathway involving the rapid capture of extracellular targets on the cell surface, however no detailed biochemical data are available. Using imaging flow cytometry and GST pulldown assays, we examined which regions of the IpLITR 1.1b CYT trigger phagocytosis and established a profile of potential intracellular signaling molecule participants. Our results show that in stably transfected AD293 cells, the membrane proximal and distal CYT regions of IpLITR 1.1b independently regulate phagocytic activities. These CYT regions differentially recruit various SH2 domain-containing intracellular mediators, providing new information about IpLITR 1.1b signaling versatility. This work sets the stage for investigating IpLITR 1.1b-mediated phagocytic signal transduction to advance our understanding of novel immunoregulatory receptor-mediated signaling events.