Conference Report: The 13th Congress of the International Society of Developmental and Comparative Immunology

L. Courtney Smith[1#], Megan A. Barela Hudgell[1], Thaddeus Deiss[2], Preethi Golconda[1], Katina Krasnec[3], Cheng Man Lun[1], Harold Neely[4], Patricia Pereiro[5], Manisha Priyam[6], Shawna L. Semple[7], Upasana Skokal[1], Luca Tacchi[3], Fumio Takizawa[8], Shruti Yadav[1], Zhen Zu[8]*

Affiliations
[1]Department of Biological Sciences, George Washington University, Washington DC, USA
[2]Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station TX, USA
[3]Department of Biology, University of New Mexico, Albuquerque NM, USA
[4]Department of Microbiology and Immunology, School of Medicine, University of Maryland, Baltimore MD, USA
[5]Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, Vigo, Spain
[6]Departmet of Zoology, University of Delhi, New Delhi, India
[7]Department of Biology, University of Waterloo, Waterloo, Ontario, Canada
[8]Department of Pathology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia PA, USA

[#]Communicating author
L. Courtney Smith, csmith@gwu.edu

*All co-authors contributed equally and are listed in alphabetical order.

The 13th Congress of the International Society of Developmental and Comparative Immunology took place in Murcia Spain from June 28 to July 3, 2015 at the Victor Villegas auditorium and Convention Center. There were two or three parallel sessions during the Congress that covered a wide range of immunological topics that brought researchers together from around the world who work in different areas of immunology. The Congress included three plenary presentations, 12 oral sessions, two poster sessions, and a special symposium. Here we report on some of the talks and a few of the posters that were presented at the meeting.

Plenary Sessions

The meeting was opened by Pablo Pelegrín (Instituto Murciano de Investigación Biosanitaria, Spain) discussed inflammasomes as key regulators of the innate immune response. Inflammasome formation is initiated by oligomerization of proteins of the NOD-like receptor (NLR) family or the NOD-like receptor with pyrin domain-containing 3 (NLRP3). NOD or NLRP3 oligomerization is activated by a variety of extracellular 'danger' signals that are detected upon infection or tissue injury, and induce inflammation with the outcome of restoring tissue homeostasis. Danger signals, or danger associated molecular patterns (DAMPs), include increased concentration of extracellular ATP, degradation of extracellular matrix components, degradation of hyaluronic acid, uric acid crystal formation, increases of reactive oxygen species, altered homeostasis, in addition to other signals. The core components of the NLRP3 inflammasome include NLRP3, apoptosis-associated Speck-like protein with a caspase recruitment domain (ASC) that functions as an adaptor to link NLRP3 to pro-caspase-1. The assembly of multiple pro-caspase 1 proteins in the inflammasome results in activation followed by cleavage of pro-inflammatory cytokines and/or leads to a pro-inflammatory form of cell death called pyroptosis. Upon infection, tissue injury or stress the cell membrane is permeabilized and ATP is released. Macrophages express the purinergic P2X7 receptor that senses extracellular ATP, induces a decrease of intracellular K^+, an increase in the production of reactive oxygen species (ROS). The resulting NLRP3 oligomerization and caspase1 activation releases IL-1β, IL-18, in addition to cathepsins, alarmins (high mobility group B1 (HMGB1) proteins), thioredoxin, caspase-1, and prostaglandins from activated cells. ASC specks released during macrophage pyroptosis may be taken up by unstimulated macrophages that induce inflammation responses in these cells and trigger inflammatory ripples that perpetuate the inflammation. Gain-

of-function NLRP3 mutations that constitutively oligomerize leads to ASC speck release, and are associated with cryopyrin-associated periodic syndromes (CAPS). CAPs patients have higher levels of ASC specks in their serum during infection compared to healthy individuals, which implies that ASC specks have potential to serve as diagnostic markers for CAPS or may be drug targets to treat these syndromes.

Gary Felton (Pennsylvania State University, USA) described herbivore gut symbionts that function at the intersection of insect and plant immunity. Herbivore/plant arms race was illustrated by saliva from a herbivore caterpillar, which was deposited onto the plant through a salivary spinneret located below the mouth and acts to suppress plant defenses. This was counteracted by the plant through detection of salivary cues to trigger plant immune responses. Alternatively, beetle herbivores regurgitate microbial gut symbionts to distract the plant from responding to damage from the herbivore. To survive, plants must differentiate between microbes vs. herbivores to activate the correct signaling pathway leading to expression of either jasmonic acid (JA) or salicylic acid (SA) hormones that mount the proper immune response. JA coordinates defense against chewing herbivores, whereas SA is responsible for defense against microbes. Negative crosstalk between JA and SA signaling is essential for plants to focus or fine-tune their immune responses against the detected threat. The major component of caterpillar saliva is glucose oxidase, which induces an oxidative burst in plant cells, activates the SA pathway and blocks the JA pathway. Regurgitated gut symbionts from the beetle and their microbial associated molecular patterns (MAMPs) results in induction of SA and suppression of JA. Induction of the incorrect plant signaling pathway drives an ineffective plant response to the herbivores, which is to the advantage of the insects.

David Schneider (Stanford University, USA) described a model that maps a path taken by a host passing through a set of disease phases beginning with infection leading to resistance (ability to clear the pathogen without signs of illness), to initial discomfort and symptoms of sickness (pathogen tolerance), which leads to either recovery or death. The model was illustrated with fruit flies infected with *Listeria monocytogenes* to explain the microbe-pathogen behavior and fly immune response and health, all in the absence of a time parameter. Similar outcomes were obtained for mice and humans when tracing disease in which a host follows a looping path through a multidimensional disease space and either returns to health through a single or multiple loops or does not complete a loop indicating termination in death. By testing the infection and

recovery parameters as well as by observing the tolerance curve using the model in resistant patients may enable a prediction of the infection-disease-recovery-or-death path for a given a patient. The ability to display the disease space visually and to see how it can be changed or warped without a time parameter can be used as a tool to follow changes and improvements for recovery from infections.

Special Symposium; Deuterostome Invertebrate Immunology

Following the 12th ISDCI Congress in Fukuoka Japan, a series of review papers on deuterostome invertebrate immunology were invited by L. Courtney Smith (George Washington University, USA) for publication in DCI (Buckley and Rast, 2015; Dishaw et al., 2014; Li et al., 2015; Pinsino and Matranga, 2015; Taketa and de Tomaso, 2015). Some of those authors were able to participate in the symposium and present an overview of their research. Jonathan Rast (University of Toronto, Canada) described conserved and divergent elements that control deuterostome immune cell development. Immune cells are ubiquitous in bilateria, but how these cell types are related based on their homology remains unclear. Although molecular similarity is obvious in genes encoding regulatory proteins, differences among the receptor and effector systems among species and cell types can be drastic and is based on rapid evolution resulting from pathogen pressure. The optimal approach for understanding evolutionary relatedness among systems that regulate cell differentiation is likely to be found at the level of gene network circuitry within cells rather than the cell types themselves. Investigations of the gene regulatory network (GRN) that controls immune cell specification and differentiation in the sea urchin embryo and larva had enabled direct comparisons to the immune cells in the well-characterized vertebrate and insect systems. Transcriptional regulatory genes correlate with multipotency and share regulators with similar systems in early hematopoietic processes in vertebrates. Genes that regulate larval immune cell specification and differentiation in sea urchins are analogous to those in vertebrates and insects. The characterization of GRNs can be used to define deuterostome immune cell development.

Katherine Buckley (University of Toronto, Canada) described gut immunity in deuterostomes using a GRN approach in a simple larval infection model. The genome of the purple sea urchin, *Strongylocentrotus purpuratus*, reveals a complex immune system that shares homologues of many vertebrate immune genes including Toll-like receptors (TLRs), NLRs,

cytokines, and transcription factors. Larvae of sea urchins are morphologically simple and transparent, which allows imaging at single-cell resolution and characterization of gene expression in sea urchin gut immunity. Exposure of larvae to live marine bacteria, *Vibrio diazotrophicus*, elicits migration of immune cells in the blastocoel to the gut, which is consistent with gene expression data. Transcriptomics of larvae responding to bacterial exposure over time identified novel effector genes, which are a set of tightly regulated genes including an IL-17 homologue that is strongly up-regulated in the gut epithelium, transcription factors (e.g. NFκB, PU.1, IRF), signaling molecules (e.g. TNF, Mif), and pattern recognition receptors (e.g. PGRPs). The use of simple sea urchin larvae illustrates the molecular complexity of inflammatory response in gut immunity, making it an ideal system to investigate this complex system that both characterizes and employs the ancient GRN of the deuterostome immune response.

Klara Stensvåg (University of Norway, Norway) described antimicrobial peptides (AMP) as host defense molecules in echinoderms. Echinoderms are known to express a wide range of AMPs that are released into the coelomic fluid by coelomocytes and function in immune defense. Two novel AMP families from sea urchin (*Strongylocentrotus droebachiensis*) coelomocytes have been identified: i) cysteine-rich strongylocins and ii) heterodimeric centrocins composed of light and heavy chains. These AMPs have conserved preprosequences, are found in both adults and pluteus stage larvae, and have potent antimicrobial properties. They are secreted by different types of coelomocytes, show different mechanisms of action with different membrane disruption mechanisms. Other AMPs have been characterized from holothuroidea including fragments of β-thymosin and the lectin CEL-III. Echinoderm AMPs are vital effector immune molecules that deter bacterial infections, and have potential for pharmaceutical applications against bacterial infections.

T cells: receptors and effector mechanisms

Several higher vertebrate species employ trans-rearrangements to diversify antigen receptors by using variable region segments from loci encoding IgM in transcripts encoding T-cell receptors (TCR). Rita Pettinello (University of Aberdeen, UK) identified a TCR complex composed of TCR chains and CD3 subunits in the small-spotted catshark (*Scyliorhinus canicula*), adding to the growing list of species utilizing a B cell-like variable segment in a TCR. The catshark has two TCRβ genes and two CD3δγ genes that were expressed in a broad range of

tissues similar to other vertebrates, with high expression in spleen and blood leukocytes. These findings suggest that a common ancestor of cartilaginous and bony fish possessed a TCR complex consisting of TCR and CD3 subunits. Thaddeus Deiss (Texas A&M University, USA) mapped the TCRδ locus in the nurse shark (*Ginglymostoma cirratum*), which has V_H segments positioned within the TCRα/δ locus, suggesting the possibility of hybrid rearrangements between the Ig and TCR loci. This result was in agreement with V_H segment usage in the TCRα/δ locus of platypus, zebra finch, *Xenopus tropicalis* and coelacanth. Although the rearrangement of V_H segments into the TCRα/δ loci was thought to occur independently in the different species, the Ig/TCRδ hybrid rearrangements using the V_H segments may contribute to TCR diversity.

Somatic hypermutation (SHM) is found exclusively in immunoglobulin genes and leads to affinity maturation of B cells in jawed vertebrates and is initiated by induced cytidine deaminase (AID). However, Michael Criscitiello (Texas A&M University, USA) described AID-induced somatic hypermutation of the TCRα chain gene in developing thymocytes of the nurse shark (*Ginglymostoma cirratum*). Although no preference for transitions or transversions was elucidated, mutation rates were similar to that for maturing B cells with significantly more mutations in the complementarity determining regions (CDRs) that form the antigen recognition site of the antigen receptor. The central thymic cortex showed the strongest expression of AID, which correlated with high expression of TCRa and β genes in the same tissue. SHM mediated by AID, active V(D)J recombination, and junctional diversification by RAG and TdT appear to be uniquely used for diversification of the primary T cell repertoire in sharks. The impact of SHM on TCR genes on T cell selection is an intriguing outcome of this novel system of diversification.

Analyses of T cell subpopulations and effector molecules in teleost fish have been dependent on the development of antibodies to identify antigen receptors and co-receptors. Teruyuki Nakanishi (Nihon University, Japan) reported that monoclonal antibodies (mAbs) to CD4-1 and CD8a in ginbuna crucian carp (*Carassius auratus*) were cross-reactive with lymphocytes from other cyprinid fish (zebrafish, common carp and goldfish) and was verified by co-staining for ZAP-70 that associates with the TcR complex during signaling. Takuya Yamaguchi (Friedrich-Loeffler Institute, Germany) developed a mAb against the rainbow trout CD8aβ heterodimer, which is a marker for cytotoxic T cells in mammals. When paired with available antibodies to CD8a in rainbow trout, Fumio Takizawa (University of Pennsylvania,

USA) showed that the majority of CD8a$^+$ cells in mucosal and systemic lymphoid organs also displayed CD8aβ although a few were negative for CD8aβ. These two cell populations were sorted and the CD8a$^+$/CD8aβ$^-$ lymphocytes had lower gene expression levels for *IFNγ* and *perforin* transcripts compared to CD8a$^+$/CD8aβ$^+$ lymphocytes. This suggested the presence of two CD8$^+$ subsets in fish; those that were CD8aa$^+$ and were either CD8aβ$^+$ or CD8aβ$^-$, in agreement with results from CD8$^+$ T cells in mice and humans.

Identification and characterization of CD4$^+$ leukocytes was reported by Kevin Maisey (Universidad de Santiago, Chile) and Fumio Takizawa (University of Pennsylvania, USA). mAbs specific for rainbow trout CD4-1 and CD4-2, which have a low level of similarity, were used to identify two lymphocyte populations in teleost fish. Some T cells co-expressed both CD4-1 and CD4-2 (CD4 DP) and others expressed CD4-2 alone (CD4-2 SP). CD4 DP lymphocytes had greater TCRβ repertoire diversity with higher proliferative responses compared to the CD4-2 SP lymphocytes. Both types of CD4$^+$ T cells produced similar levels of Th1 and Th17 cytokines in response to *Yersinia ruckeri* infection. Some rainbow trout macrophages expressed CD4-1 and were active phagocytic cells, whereas CD4$^+$ lymphocytes were not. However, a polyclonal antibody (pAb) against rainbow trout CD4-1 identified CD4-1$^+$/CD3ε$^+$ lymphocytes from rainbow trout spleen that were phagocytic, which could be increased by stimulation with IL-2, IL-15 and IFN-γ. CD4 expression in trout lymphocytes and macrophages can be used to identify additional subsets of cells.

Zebrafish are an excellent model for investigating development and for live imaging of the immune system. However transgenic zebrafish with identifiable T cell subpopulations have been unavailable. Christopher Dee (University of Manchester, UK) successfully generated CD4-1 transgenic zebrafish and showed that fish expressing both *cd4-1:mCherry* and *lck:GFP* identified lymphocytes with T helper cell features, in addition to macrophages that only expressed mCherry. This advance is expected to become a highly useful tool for visualization of the behavior of CD4$^+$ T cells and macrophages *in vivo*. In other work with zebrafish, Beatriz Novoa and Patricia Pereiro (Instituto de Investigaciones Marinas, Spain) reported (respectively) the identification of *perforin* (*prf*) and *NK-lysin* (*nkl*) genes. Expression of six *prf* and four *nkl* genes showed tissue, cell type and developmental stage specific expression patterns, suggesting distinct functions for perforins and NK-lysins. Some isoforms of NK-lysin genes (*nkla* and *nkld*) and most of *prf* genes were significantly induced in the kidney of adult zebrafish upon injection with

spring viremia of carp virus (SVCV). Expression of *pfn19b* (likely an orthologue of mammalian and shark *prf*) was increased in zebrafish larvae in response to SVCV infection, and overexpression was protective when associated with the expression of *caspase a* and *IL-1β* and increased caspase-1 activity. Higher expression of a constitutive *nkl* in turbot (*Scophthalmus maximus*) correlated was associated with greater resistance to viral hemorrhagic septicemia virus (VHSV). Consequently, *nkl* may be useful as a transgene to generate a line of fish that are more resistant to viral diseases.

Fish B cells and Immunoglobulins

Zhen Xu (University of Pennsylvania, USA) reported that although IgM is the predominant immunoglobulin in the gill mucous of rainbow trout, IgT levels were remarkably high in gills relative to other tissues. The majority of bacteria isolated from gill mucous were coated with IgT and were associated with the poly-Ig receptor (PolyIgR; an immunoglobulin transport protein), which was expressed by the gill epithelium. Most interestingly, during an acute immune response to *Ichthyophthirius multifiliis* (or Ich infection), proliferation of IgT^+ B cells was detected in the gills but not in the spleen. Parasite specific IgT was produced locally in gills, while anti-parasite IgM was detected almost entirely in the serum. Oriol Sunyer (University of Pennsylvania, USA) described a novel method for evaluating the importance of IgT by depleting IgT^+ B cells in the rainbow trout. Fish were injected with a mouse monoclonal antibody (mAb) to IgT, followed by a trout Ab against the mouse antibody. This ablated 95%of the IgT^+ B cells without any changes to the levels of the IgM^+/IgD^+ B cells. These fish were used to evaluate the importance of mucosal IgT in both pathogen clearance and maintenance of microbiome homeostasis. Results from fish with ablated IgT^+ B cells showed altered the microbiome at the gill surface plus dramatic changes in the microbiome of the skin. IgT^+ B cells were protective against the bacterial pathogen, *Flavobacterium columnare*, and Ich infection, based on increased mortality and increased pathogen load in fish depleted of IgT^+ B cells. These results demonstrated the importance of IgT in fish in pathogen control and clearance at mucosal surfaces, in addition to the IgT involvement in maintaining the homeostasis of microbiota.

The primordial secondary lymphoid tissue, the spleen, arose at the dawn of adaptive immunity and is the centralized location for germinal center formation in mammals. Other vertebrate species such as the nurse shark (*Ginglymostoma cirratum*) and the frog (*Xenopus*

laevis) have a spleen, but lack canonical follicular dendritic cells (FDC) that are required for germinal center formation in mammals. However, B cells from *X. laevis* undergo class switch and somatic hypermutation of the Ig loci, which are mechanisms generally associated with the formation of germinal centers. Harold Neely (University of Maryland, USA) showed that XL cells in *X. laevis* have functions similar to FDCs and express proteins such as CXCL13, BAFF, and PolyIgR2, which are important in recruiting and positioning lymphocytes as well as stimulating the adaptive immune system. Results suggest that XL cells *X. laevis* may act as FDCs to organize a primordial type of germinal center.

MHC and Antigen Presentation

Yuko Ota (University of Maryland, USA) presented evidence from genomic analysis suggesting that canonical MHC and immunoglobulin based antigen receptor genes shared a common ancestor. Primordial immune genes were tracked in the *Xenopus laevis* genome, a species from the dawn of the second round whole genome duplication (2R). Genome duplication provides opportunities for functional divergence of the expanded members of a gene family including silencing and translocation to other regions of the genome. The proto-MHC genes of *X. laevis* are positioned in four paralogous regions of the genome as a result of the 2R duplication event. One region has a fused immunoglobulin VJ segment and a C region exon near the proto-MHC locus. The VJ and C segments potentially predate the appearance of antigen receptors and provide evidence of common ancestry for the MHC and immunoglobulin adaptive immune receptors. However, fused VJ segments have also been identified that predate the 1R duplication event suggesting the opposite outcome; that antigen receptors predate the appearance of MHC.

Mucosal Immunity

Mucosal immunology is characterized by investigations of structures, cells, proteins, and compounds that make up the mucosal barrier found in the gut, nasal cavities and, in the instances of fish, this includes the gills and the skin. The fish mucosal immune system can be categorized as complex lymphoid tissues that express IgM, IgD, IgT and/or IgZ, and the PolyIgR, include B cells, and act as a mucosal barrier between the epithelial cells and the environment. Lucca Tacchi (University of New Mexico, USA) described lymphocyte-rich lymphoid aggregates

(LAs) in the nasal and intestinal mucosa of the African lungfish (*Protopterus dolloi* and *P. annectens*). In response to LA up-take of antigens via mechanisms similar to those described in mammals (which include dendritic cells and migrating microphages), expression of multiple immunoglobulin isotypes, lymphotoxin and other TNF superfamily members were detected. Bacterial infection resulted in structural changes in the LAs with a dramatic increase of T and B cells. The *de novo* appearance of inducible LAs (iLAs) was observed, suggesting a mechanism of organogenesis remarkably similar to that of mammalian tertiary lymphoid tissues. Results suggested conserved functions of LAs and specialized mucosal immunity that appeared early during the evolution of the tetrapods.

Mucosal-associated lymphoid tissues (MALTs) contain B cells and express immunoglobulins that function in the maintenance of mucosal homeostasis. Although the structure and functions of mucosal secreted IgA (sIgA) have been well described in higher vertebrates, functions of mucosal B cells and immunoglobulins in teleost fish is not well understood. However, Erling Olaf Koppang (University of Life Sciences, Norway) who showed B and T cells of Atlantic salmon (*Salmo salar*) were present in the interbranchial lymphoid tissue (ILT) of the gills, which is a site of substantial antigen/pathogen exposure and has a structure consistent with a secondary lymphoid organ, perhaps equivalent to mammalian MALT. Irene Salinas (University of New Mexico, USA) described the nasopharynx-associated lymphoid tissue (NALT) of trout, which is similar to other teleost MALT based on the distribution of B cell subsets producing sIgs and the presence of sIg-coated microbiota. Trout NALT showed higher expression of IgT compared to other tissues, and function in microbiota control (see report for O. Sunyer above). The distribution of teleost IgT^+ B cells in NALT led to the question of producing nasal vaccines using salmonids as a model organism for nasal vaccine research. Intranasal vaccination with enteric redmouth disease (ERM) and infectious hematopoietic necrosis virus (IHNV) into separate nares (nostrils) provided a higher level of protection against these two pathogens compared to the combined vaccine. Using this intranasal immunization protocol, Cecelia Kelly (University of New Mexico, USA) analyzed the kinetics and transcriptional regulation of the innate immune response genes in the nares and showed a rapid upregulation of IL-1β and CCL19, among other genes. Intranasal immunization elicited a more robust and rapid response compared to intramuscular and immersion immunizations. In general, separate nares vaccine administration may prove to be a useful tool for the aquaculture industry for maintenance of farmed salmon.

Antiviral Immunity; IFN and IFN-induced genes

Jun Zou (University of Aberdeen, UK) reviewed the origin and evolution of the interferon (IFN) system in jawed vertebrates and introduced the three IFN families: Types I, II, and III. Cartilaginous fish (e.g., the elephant shark, *Callorhinchus milli*; the small-spotted catshark *Scyliorhinus canicula*) have complex type I and III IFN systems that primarily have innate immune function through induction of an antiviral state, and were expressed in response to poly I:C. Type I IFN gene structure in cartilaginous fish appeared more complex than homologous genes in higher vertebrates because they include introns. Type I IFN genes of higher vertebrates are intronless and may have resulted from retrotransposition of a transcript into the locus at some point after the divergence of reptiles and bony fish. These studies shed new light on the origin and evolution of IFN system in vertebrates.

The IFN signaling pathway in the European seabass (*Dicentrarchus labrax*) was described by Yulema Valero (Instituto Español de Oceanografía, Spain), which included MDA5, LGP2, MAVS, TRAF3, TANK, TBK1, IRF3, IRF7 and PKR. Most of the genes encoding these proteins were induced in the seabass gonad in response to infection with a nodavirus (VNNV), and MDA5, LGP2 and IRF3 were also induced in the brain. The outcome of the IFN signaling activates Interferon regulatory factors (IRFs) that regulate the expression of IFN and IFN-stimulated genes (ISGs) and Qiwei Qin (Chinese Academy of Science, China) described two novel IRFs (EcIRF3 and EcIRF7) from the grouper (*Epinephelus coiodes*). EcIRF3 expression was important for responses to red spotted grouper nervous necrosis virus (RGNNV), whereas EcIRF7 inhibited replication of the Singapore grouper iridovirus (SGIV). Other proteins involved in the IFN pathway include tripartite motif-containing (TRIM) proteins and Christelle Langevin (French National Institute for Agricultural Research, France) described certain TRIMs that are conserved among fish including the sea bass (*Dicentrarchus labrax*), rock cod (*Trematomus bernacchii*) and icefish (*Chionodraco hamatus*). One TRIM from zebrafish, called av-ftr, was expressed in gills and pharynx, activated the type I IFN pathway, induced IRF3, and was protective against RNA viruses.

Members of the Suppressor of Cytokine Signaling (SOCS) family down-regulate cytokine activities. SOCS1, 3, 5, 6 and 9 and CISH identified by Kittipong Thanasaksiri (Tokyo University of Marine Science and Technology, Japan) were expressed in all tissues examined from the Japanese flounder (*Paralichthys olivaceus*). Fish stimulated with poly I:C induced an

over expression of SOCS1, whereas SOCS1, SOCS3 and CISH were up-regulated after fish were infected with the bacteria, *Edwarsiella tarda*. Results suggested that SOCS1, SOCS 3 and CISH have different regulatory functions in immune responses against different pathogens in the Japanese flounder.

There are many bacterial and viral pathogens of aquatic organisms and concurrent vaccination with both bacterial and viral antigens would seem optimal and sensible. Despite the practicality of simultaneous vaccination, Helle Kristiansen (Aarhus University, Denmark) suggested that this may actually inhibit immunological function in rainbow trout. Intramuscular (im) injections of a DNA vaccine containing the gene for the viral hemorrhagic septicemia virus (VHSV) glycoprotein resulted in protective immunity that was primarily attributed to the up-regulation of IFNs and IFN induced genes such as Mx. When fish were given a simultaneous vaccination against VHSV plus bacteria to determine whether this would interfere with protective immunity induced by VHSV vaccination, fish were not well protected against challenge with VHSV eight days after receiving both vaccines, although protection improved by 11 weeks. Mx expression was significantly decreased in fish receiving both vaccines suggesting that the bacterial vaccine negatively impacts the IFN response, similar to results in mammals. The results stress the importance of additional studies to evaluate the efficacy of simultaneous vaccination procedures in an aquaculture setting.

MicroRNAs (miRNAs) are short non-coding RNAs that regulate gene expression in eukaryotic cells by binding to target sites in mRNAs to block transcription or induce mRNA cleavage. Niels Lorenzen (Aarhus University, Denmark) showed that two teleost miRNAs, miR-462 and miR-731 involved in anti-viral immune response were up-regulated in rainbow trout inoculated with the viral hemorrhagic septicemia virus (VHVS) or with poly I:C. Injection of complementary miRNAs immediately after poly I:C challenge reduced the protective effect of poly I:C against VHVS suggesting that miR-462/731 have core activities in the IFN mediated anti-viral immune response in teleost fish. miR-462/731 are orthologues of human miR-191/425 that are involved in cell cycle control, therefore it appears that evolution may have led to different functions for genetically related miRNAs clusters.

Host Microbe Interactions

Maricultured invertebrates require the development of vaccines to improve aquaculture farm production. White spot syndrome virus (WSSV) is a potent pathogen of penaeid shrimp and an outbreak can kill an entire shrimp population. Han-Ching Wang (Shandong University, China) showed that WSSV manipulated long chain fatty acids of the host crustacean through PI3K-Akt-mTOR-HIF1a pathway. WSSV induced an invertebrate Warburg effect, or an increased rate of glycolysis, during the genome replication stage that led to increased cell energy usage and the biosynthesis of macromolecules including converting the process of lipolysis to lipogenesis, all of which benefited viral replication. WSSV also interfered with sequestration of iron by apoferritin to form ferritin, which is a normal host mechanism to reduce nutrients for pathogens. The interaction of the viral serine/threonine protein kinase (PK1) with shrimp ferritin altered iron binding by apoferritin and elevated levels iron available for virus replication. WSSV attacks the shrimp cells in multiple ways to enable effective replication and each suggests a means for producing vaccines.

During times of nutrient starvation, cells activate autophagy to degrade unnecessary cellular proteins to recycle the resulting components for constructing macromolecules critical for survival. Because some viruses exploit autophagy as part of their life cycle, Phuc Pham (University of Waterloo, Canada) used two fish cell lines (a monocyte/macrophage cell line derived from rainbow trout spleen (RTS-11), and an epithelial cell line obtained from fathead minnow (EPC)) to investigate the relationships among starvation, autophagy and viral infections. Cells were exposed to two viruses, Viral Hemorrhagic Septicemia Virus IVa (VHSV) and Frog Virus 3 (FV3) and starvation in low nutrient media resulted in lower replication of VHSV. However replication of FV3 was unrelated to whether cells were starved or not. These results suggested that during times of starvation when cellular processes are either down-regulated or are limited, some viruses are still able to manipulate cellular machinery to ensure their propagation.

Fish Models of Immunity and Inflammation

Most organisms rely solely on their innate immune systems to survive infections. Although fish that have both innate and adaptive immunity, loss of the adaptive system would hypothetically induce compensation by the innate mechanisms. To test this, Alicia Martinez-

Lopez (Miguel Hernandez University, Spain) subjected wild-type and RAG1 mutant zebrafish (*Danio rerio*) to infection with spring viremia of carp virus (SVCV) and observed that mutant fish had higher resistance to viral infection compared to wild-type fish. Results suggested the involvement of several multi-gene families that function in viral resistance and indicate unknown levels of interplay between the innate and adaptive immune systems. Outcomes are likely to lead to improved design of viral vaccines and adjuvants for fish in the future.

Growth factors such as progranulins (PGRNs) and insulin-like growth factors (IGFs) are necessary for cell survival and proliferation. Hong-Yi Gong (National Taiwan Ocean University, Taiwan) identified PGRN2 in Mozambique Tilapia (*Oreochromis mossambicus*) and showed that infection with either Gram negative *Vibrio vulnificus* or Gram positive *Streptococcus iniae* could induce expression of PGRN2 in immune-related tissues such as the head-kidney, spleen and intestine. Transgenic zebrafish expressing a short version of the PGRN2 gene induced expression of inflammatory cytokines and enhanced survival rates upon infection by *Vibrio vulnificus*, thus concluding that this novel Tilapia short PGRN2 gene could be used to modulate innate immunity in zebrafish.

Shawna Semple (University of Waterloo, Canada) described how family cohorts of rainbow trout (*Oncorhynchus mykiss*) could be used to develop a vaccine against infectious bacteria such as *Flavobacterium psychrophilum*. Some sets of full siblings were resistant and/or susceptible to bacterial challenge, and resistance was transferable to the next generation without negative effects on growth and development of the fish. When either resistant or susceptible siblings were challenged with *F. psychrophillum*, higher levels respiratory burst activity in head kidney leukocytes may be the crucial clearance mechanism for fish survival of bacterial cold-water disease (BCWD). Differences in immunological functions for resistant vs. susceptible trout may provide essential information for the development of future vaccines.

BCWD in young fish leads to high mortality and great economic losses for aquaculture. There is no effective vaccine for BCWD, however Gregory Weins (United States Department of Agriculture, USA) focused on selectively breeding populations of rainbow trout that survive BCWD and understanding the pathophysiological mechanisms of survival. Fish tend to exhibit two primary defense strategies against bacterial infections, i) resistance, which is the ability of the host to block pathogen proliferation, and ii) tolerance, which is the host ability to limit the negative health impacts of a pathogen load. Selectively bred rainbow trout with high and low

survival against BCWD were stocked and evaluated in aquaculture settings. Farms stocked with high performing fish did not have an outbreak of BCWD, whereas about half of farms stocking the low performing fish had outbreaks. Preliminary analyses of markers for fish tolerance to bacterial load suggest that although this was not the basis of survival against BCWD, resistance was a heritable mechanism that enabled fish to combat effectively this bacterial infection.

Omics in comparative immunology

Next generation sequencing and transcriptome profiling provides a global analysis of host responses to pathogens. Diego Valenzuela-Miranda (University of Concepcion, Chile) employed RNAseq to unravel the mechanism by which the Infectious Salmon Anemia Virus (ISAV) induces changes in gene expression in tissues from the Atlantic salmon, *Salmo salar*. Although salmon show a strong immune response to infection by ISAV, they eventually succumb to the infection. Fish infected with the virus were used to correlate viral replication with host immune response in head-kidney, liver and gills. Changes in gene expression showed elevated viral replication in the liver with an abundance of viral sequence segment 7 that was associated with divergent IFN expression and antiviral activities. The transcriptional response in the head-kidney and gills was linked to an inhibition of endocytosis plus elevated levels of mRNAs encoding proteins involved in the inflammatory response.

In an investigation of obesity and metabolic syndrome related diseases, such as non-alcoholic fatty liver disease (NAFLD) in zebrafish (*Danio rerio*), Antonio Figueras (Instituto de Investigaciones Marinas, Spain) evaluated the liver transcriptome profile in diet induced obese fish to determine how obesity affected the immune response to infection. Overfed fish showed signs of liver steatosis (fat accumulation), similar to observations in humans, however no differences in gene expression were observed in overfed fish stimulated with LPS compared to over-fed, non-stimulated controls. Conversely, healthy fish responding to LPS showed a change in gene expression in response to immunological challenge and a typical host defense reaction that was similar to that observed in mammals. This suggested that liver damage and altered metabolism in obese fish undermined responses to infection. Furthermore, similarities in the gene expression profile between fish and humans may be employed to identify pathological markers for liver malfunction in humans. David Raftos (McQuarrie University, Australia) described a meta-analysis of data collected from 14 studies on the transcriptomes of several

oyster species under both pathogen challenge and environmental stresses. The pattern that emerged from 586 genes that were either up- or down-regulated identified 12 discrete cellular processes regardless of whether responses were to bacterial or parasitic pathogens (viruses induced a different response) or to stress. Changes in gene expression were similar for outbred oysters and for those selected for pathogen resistance, and outcomes repeatedly pointed to the involvement of increased energy demand and oxidative stress. Results pointed to predictive markers for oysters that correlated with a 30% increased survival rate. In addition to the identification of stress response genes, this meta-analysis identified lineages of oysters that could be selectively bred to respond to or tolerate stress, suggesting they will also show increased resistance to microbial pathogens and parasites. This new information is expected to be important for the shellfish aquaculture community and for an economically important species of the food industry.

Pattern Recognition Receptors

Genes encoding Down Syndrome Cell Adhesion Molecules (DSCAM), previously identified in *Drosophila*, *Daphnia*, Tiger shrimp, and the shore crab, *Carcinus maenas*, encode three highly variable immunoglobulin domains, Ig2, Ig3, and Ig7, two transmembrane regions, plus multiple possible 3' untranslated regions. Published results from conventional sequencing of DSCAM mRNAs from the mosquito showed that the variable domains were alternatively spliced in response to the type of immune challenge providing a degree of specificity to the pathogens. However, conventional sequencing may not provide enough coverage to demonstrate how the transcription and splicing of Ig domains may change during the course of an infection. Chris Hauton (University of Southhampton, UK) used unbiased, high throughput sequencing to examine transcription and splicing changes in hemocytes from the shore crab, *Carcinus maenas*, after stimulation with Gram positive bacteria and although no increase in the expression of DSCAM was found, new exons were used for the Ig7 region. It was noteworthy that DSCAM from different crabs showed individual-specific splicing for the Ig7 region, perhaps because Ig7 may not be directly involved in interactions with pathogens, and that splicing variations for Ig3 and Ig3 domains may show different results.

Immune priming that infers non-self recognition specificity in innate immunity is a relatively recent concept in the field of immunology, although its existence was assumed and

evidence was sought during the 1970s and early 1980s. The notion of innate immune specificity lost favor in the mid 1980's, but has recently been resurrected as the concept of trained immunity. Tze Hann Ng (National Cheng Kung University, Taiwan) reported that DSCAM is a key molecule in specific immune memory in crayfish (*Cherax quadricarinatus*). Two versions of DSCAM are expressed in crayfish; cell surface membrane-bound forms and "tail-less" or secreted forms that are present in the hemolymph after pathogen challenge. The question addressed was whether pathogen challenge could drive the generation of different versions of DSCAM that were specific for binding to or providing immune protection from the inducing pathogen. DSCAM proteins were isolated from crayfish that survived for two months after infection with the White-spot syndrome virus (WSSV) and after re-challenge. DSCAM mRNA isolated from hemocytes after the first and second challenge showed that sequence diversity increased in the Ig2 and Ig3 variable exons of WSSV-induced DSCAM isoforms after the first challenge, but decreased after the second challenge to encode the "correct cloud" of exon usage. The long-term crayfish survivors of the initial infection were those that spliced the correct DSCAM cloud of exons using an unidentified mechanism for protection against re-encountered WSSV. DSCAM proteins with the correct cloud of domains showed high WSSV binding ability and specific immune protection against WSSV. Although the binding specificity only lasted for two months, these results were an important step in the development of vaccines against WSSV.

Annelid species including earthworms use innate immunity for protection against both ingested and environmental pathogens, and employ Toll-like receptors (TLRs) that fall into two categories; the "vertebrate-like" (V-type) and "protostome-like" (P-type). Radka Roubalova (Academy of Sciences, Czech Republic) described the TLRs in the earthworm that included V-type TLRs with a cysteine-rich N-terminus, substantial variability in the LRR sequences that followed, and a C-terminal leucine rich repeat motif. The P-type TLRs have LRRs that are interrupted with LRR-NT and LRR-CT motifs and had low sequence diversity. Both types of TLRs were induced by contact with Gram negative bacteria. The V-type TLRs showed highest expression in the intestine, protosome, gizzard, crop, and esophagus, whereas expression of the P-type TLRs was present in the anterior digestive tract and clitellum with highest expression in the cocoon and lowest in adults. The overall lack of variability of the P-type TLRs in the earthworm along with its decline in expression levels in adults suggested that the P-type TLRs may function more in development, while V-type TLRs may function in immunity.

The lipopolysaccharide-binding protein (LBP) and bactericidal/permeability-increasing protein (BPI) are structurally related proteins in mammals that have antagonistic activities and provide protection against bacterial pathogens. These proteins have also been found in non-mammalian vertebrates and invertebrates, but because of the similarities between LBP and BPI homologues cannot be classified as one or the other. Petra Prochazkova (Academy of Sciences, Czech Republic) reported a complete cDNA sequence of an LBP/BPI homologue from the earthworm *Eisenia andrei*, which has two conserved domains with putative LPS binding function. The gene showed constitutive expression in all tissues but was highly expressed in the pharynx, esophagus, coelomocytes, and seminal vesicles. The earthworm LBP/BPI homologue appeared to be a pattern recognition receptor for LPS and could be induced in coelomocytes in response to Gram positive and Gram negative bacteria. Martin Bilej (Academy of Sciences, Czech Republic) described on the importance of mucosal immunity in the earthworm, *Eisenia andrei*, in which non-commensal bacteria are present in the coelomic cavity and along the intestinal lining. The phagocytic cells in the earthworm accounted for the massive influx of bacteria, which were brought into the coelom as a result of changes in pressure gradients that enable worm mobility. The large numbers of microbes in the ingested soil correlates with the expression of PRR families in the cells that line the gut and include LPB binding proteins, coelomocyte cytolytic factor, and TLRs. These function in surveillance of bacterial infiltration into the gut tissues and the induction of effector activities, which are modulated based on the microbial challenge.

TLRs remain a crucial aspect of both vertebrate and invertebrate innate immunity. In birds, the pattern of TLR evolution is critical for understanding avian immunogenetics and the ecology of infectious diseases. Michal Vinkler (Charles University, Czech Republic) reported on TLRs in the passerine and Galloanserae groups, which included TLR4 that recognizes complex ligands, TLR5 that binds flagellin, and TLR7 that detects ssRNA. These three TLRs were chosen for analysis because their ligands differ from homologues in fish and mammals. TLR4 and TLR5 had sequence variations among bird species with variant positions near the ligand binding sites. However, the significant sequence variability within TLR4 and TLR5 that detected bacteria was not observed for viral-sensing TLR7, revealing a marked distinction between bird TLR7 and homologues in fish and mammals. The identification of positive selection and sequence variability in the functional regions of bird TLRs suggested that pathogen

mediated selection may have impacted the PRRs differently in avian groups compared to fish and mammals.

The differences in pathogen resistance and diversity in the innate immune system in agricultural avian species and those of fancy breeds may lead to the identification of a more disease-resistant bird. Zuzana Bainova (Charles University, Czech Republic) examined the sequence diversity of four bird TLRs (TLR3, TLR4, TLR5, and TLR7) among 25 chicken breeds in Europe. The largest number of single nucleotide polymorphisms was present in TLR3, but all TLRs showed some degree of variability. Although the majority of the substitutions were not near the ligand binding regions, a few sites in TLR4 and TLR7 were near functionally important sites and were under positive selection. A computational network showing relationships among haplotypes from all sequences showed that many protein variants tended to cluster together, yet some alleles were present across the network. Greater gene flow and polymorphism were present in the fancy chicken breeds compared to domestic chickens, suggesting that the introduction of some fancy breed TLR variability into domesticated chicken lines may reduce mortality of agriculturally raised chickens.

TLRs are critical for initiating immune responses through ligand recognition and signaling leading to the nuclear localization of NFκB, which is essential for immune gene transcription, cytokine production, and cell survival. Although TLRs are well documented in birds, mammals, amphibians and fish, very little is known about TLRs in reptiles. Carlos Voogdt (Utrecht University, The Netherlands) described evidence for TLRs in the green anole lizard, *Anolis carolinensis*. Using the green iguana cell line, a NFκB luciferase reporter assay was used to show that NFκB production increased when the cells were stimulated with various TLR ligands. The greatest response was observed for flagellin from *E. coli* (FliC) suggesting the presence of TLR5. When the genome for *A. carolinensis* was searched, a putative TLR5 gene (*AcTLR5*) was identified, and expression was verified in several reptilian tissues using RT-PCR. When recombinant *AcTLR5* was expressed in the iguana cell line, response to FliC resulted in a significant increase in NFκB expression. When comparing reptilian and human TLR5 responses to flagellated microbes, AcTLR5 only recognized the reptilian pathogen, *Pseudomonas aeroginosa*, whereas the human TLR5 only recognized the human pathogen, *Salmonella enterica*. TLR specificity for ligand binding among different species may provide a more

complete picture of TLR function through vertebrate evolution including the assumption that these PRRs are generally conserved among vertebrates,

TLR9 recognizes unmethyled CpG DNA, a PAMP for bacteria and DNA viruses. Fang-Yao Lee (Academia Sinica, Taiwan) described two isoforms of TLR9 from groupers (*Epinephelus spp*) encoded by the *gTLR9* gene; the full-length version (gTLR9A) and a truncated version that was missing the C-terminal TIR domain (gTLR9B). The rapid transition of expression from *gTLR9A* to *gTLR9B* suggested changes in alternative splicing, and hypo- vs. hyper-phosphorylation of Pol II that determined splicing transition for the *gTLR9* transcripts from *A* to *B* isoforms. Manipulation of the p-TEFb kinase that phosphorylated the Pol II C-terminal domain, which also interacted with NFκB, changed the overall alternative splicing strategy of *gTLR9*. This suggested that NFκB regulated the *gTLR9* alternative splicing via the level of Pol II C-terminal domain phosphorylation. The identification of TLR9B in other closely related fish species suggested that this alternative splicing dependent switch may not be grouper specific and may be an evolutionarily conserved measure in teleosts for generating TLR9 responses

Vertebrate NOD-like receptors (NLRs), the adaptor protein (apoptosis-associated speck-like protein containing CARD (ASC)), plus caspases multimerize and interact to form an inflammasome when appropriate PAMPs are detected. There were 13 predicted NLR gene sequences (NLRC1 to NLRC12 and NLRX3) identified from the Japanese pufferfish (*Fugu rubripes*) genome. Jun-Ichi Hikima (University of Miyazaki, Japan) used the signaling cascade mediated by inflammasomes in *Fugu* to identify the NLR genes that were induced by inflammasome formation in fish. LPS stimulation of pufferfish head kidney (HK) cells resulted in up-regulation of NLRC1, 5, 7, 9, 10 and 12, while *in vitro* treatment with nigericin, an antibiotic that induces inflammasomes, plus a combination of LPS and nigericin resulted in increased expression of NLRC10 and 12 in HK cells. Nigericin also stimulated HK cells and leukocytes to increase transcription of caspase-1, ASC, IL-1β, and IL-18, and was confirmed by enhanced superoxide anion production, phagocytic and lysozyme activities. These results indicate that *Fugu* innate immunity includes an inflammasome mediated cytokine response.

Receptor-interacting serine/threonine kinase 2 (RIP2) is a CARD containing protein that functions in the signaling pathways of various PRRs. In mammals, RIP2 initiates a response generated by NOD1 and NOD2, however the functions of RIP2 in fish are relatively unexplored.

Jiasong Xie (University of Alberta, Canada) characterized RIP2 in goldfish that showed elevated gene expression in spleen, monocytes, and splenocytes. RIP2 function in monocytes could be inhibited by blocking the MAPK pathway and activated by live *Mycobacterium marinum* and several PAMPs. Co-immunoprecipitation identified interactions with both NOD1 and NOD2, whereas overexpression of RIP2 resulted in activation of the NFκB signaling pathway and production of TNFα and IL-1β1. These results show that RIP2 has pivotal activities in goldfish immunity against *M. marinum* through its involvement in immune signaling pathways.

Lectins

Lectins that bind to carbohydrates on cell surfaces are implicated in commensalism, symbiosis, host-colonization, pathogen recognition and "subversion" of pathogen recognition by the immune system. There are a variety of structural folds in lectins that define their category; C-type, galectins, F-type, P-type I-type and pentraxins. Gerardo Vasta (University of Maryland, USA) described F-type lectins that bind fucose, have a unique lectin fold, and are found in a wide range of organisms including bacteria, invertebrates and vertebrates. Two examples of F-type lectins identified in the European eel *Anguilla anguilla* (AAA) and the striped bass *Morone saxatilis* (MsaFBP32) led to the establishment of the new animal F-type lectin family. The protein structure of F-type lectins showed a wide range of domain organization with single to multiple tandem carbohydrate recognition domains (CRDs) in association with other types of domains. AAA was composed of a single CRD that bound Ca^{2+} and the sugar ligand, whereas MsaFBP32 had two CRDs that were structurally quite different and showed binding specificities towards different glycans. The CRDs were positioned at the opposite ends of the protein, which associated into dumbbell-shaped trimers. MsaFBP32 may mediate opsonin and phagocytosis functions by cross-linking microbes and host phagocytes. Diversity in carbohydrate recognition by F-lectins as determined by their tandem CRDs may result in a broad range of functions including both self and non-self recognition to induce phagocytosis in addition to non-immune functions such as fertilization and other biological activities.

Gerardo Vasta also described two galectins (CvGal1 and CvGal2) expressed by the hemocytes of the eastern oyster (*Crassostrea virginica*). In addition to their activities in cell-cell and cell-matrix interactions (self-recognition), galectins recognized glycans on viruses, bacteria, fungi, and parasites (non-self recognition). They also bound to trophozoites of the protozoan

parasite, *Perkinsus marinus*, which was dose-dependent and based on β-galactoside specificity. Trophozoites were opsonized by CvGal1 and phagocytosed by oyster hemocytes. Once within the hemocyte, parasites proliferated and hijacked the cells to spread them throughout the body of the oyster, which led to systemic infection and death. On the other hand, negligible binding was observed between CvGal1 and *P. chesapeaki*, a sympatric parasite species prevalent in clams. Phylogenetic analysis showed that CvGal1 and CvGal2 had similar CRD structures, yet results from analysis with a glycan array indicated broader binding specificity and different binding kinetics for CvGal2. Structural modeling of these galectins identified significant differences in their sugar binding interactions, which suggested that that *P. marinus* has developed carbohydrate based mimicry of the host endogenous ligands. The parasite may bind these galectins to induce uptake into the hemocytes and avoid host immune attack. Furthermore, because the galectins bound poorly to *P. chesapeaki*, this was a likely basis for why the parasite does not infect *Crassostrea virginica*.

Complement

The complement system is a set of proteins in both vertebrates and invertebrates that function in tagging pathogens for phagocytosis plus, in vertebrates, for microbial lysis. Helen Dooley (University of Aberdeen, UK) discussed the evolution of haptoglobin (Hp) and the mannose binding lectin-associated serine protease (MASP) family in the nurse shark (*Ginglymostoma cirratum)*. Hp in mammals is an acute phase plasma protein that binds irreversibly with high affinity to hemoglobin (Hb) released by intravascular hemolysis. The Hp:Hb complex is cleared and degraded by the macrophage scavenger receptor CD163, which reduces Hb-induced oxidative damage to host cells and tissues. In the nurse shark, the Hp orthologue did not bind Hb, which was based on missing loop 3 in the protein that was deemed important for binding to Hb and uptake by CD163. Hp sequence comparisons showed that loop 3 was absent in all vertebrates except mammals suggesting that Hp binding to Hb is a relatively recent evolutionary event. Phylogenetic analysis illustrated Hp to be an ancient member of the MASP family of proteins, which included key initiators of the complement system (MASP1, MASP2, MASP3, C1r and C1s) and may have arisen from independent duplication events. Similarly, the assembled transcriptome of the small-spotted catshark, *Scyliorhinus canicula*, reported by Anthony Redmond (University of Aberdeen, UK) was highlighted by a detailed

phylogenetic analysis of the fast evolving MASP gene family. Results confirmed the identification of the shark complement homologues, and demonstrated that sharks were missing MASP2 while teleosts were missing MASP1.

A unique feature of the teleost complement system is that the components have been diversified into multiple isoforms with different functions. Properdin (Pf) is an important positive regulator that stabilizes C3 convertase and acts as a pattern-recognition molecule to trigger the alternative complement pathway (ACP). Miki Nakao (Kyushu University, Japan) presented the structural and functional diversity of properdin isotypes in the common carp, *Cyprinus carpio*. Two similar cDNA sequences of carp Pf shows expression in different tissues; CaPf1 in spleen, and CaPf2 in the kidney. The two Pf proteins form hexamers in the serum, have different binding specificities for various microbes, and have different abilities for binding the variants of teleost complement C3 proteins. These results illustrated the diversification of carp properdins that has expanded the complexity of the teleost complement system.

Invertebrates also have complement proteins with opsonization activities. Petr Kopacek (Academy of Sciences, Czech Republic) focused in the functions of two putative complement C3 convertases, IfFC and IfC2/Bf, from the hard tick, *Ixodes ricinus*. This arthropod is a European vector for transmission of a large variety of pathogens that cause diseases such as Lyme borreliosis, tick-borne encephalitis virus, and human granulocytic anaplasmosis. Although the tick is a difficult experimental animal, *I. ricinus* has great potential for reverse genetics and functional genomics. The IrC2/Bf convertase was expressed in trachea and fat body and IrFC was expressed in hemocytes. IrFC was involved in augmenting phagocytosis of Gram negative bacteria, whereas IrC2/Bf functioned in phagocytosis of yeast and *Borrelia*. Down-regulation of IrC2/Bf resulted in reduced phagocytosis of *Borrelia*, however, expression of IrFC compensated for this reduction. IrC2/Bf expression was responsive to challenge with yeast and *Borrelia*, likely resulting from TLR signaling, whereas IcFC expression was responsive to injury, suggesting activities in wound healing. These results indicated the presence of at least two complement-like pathways in *I. ricinus* in which IrFC and IrC2/Bf acted as specific convertases of different C3 complement components.

Antimicrobial Responses

Audrey Majeske (University of Puerto Rico, USA) described the expression of *Sp185/333* immune genes in phagocytes of the purple sea urchin, *Strongylocentrotus purpuratus*. Previous studies have characterized the expression patterns of this gene family in response to pathogen or PAMP challenge in a variety of tissues and cell populations but not in single phagocytes. Upon challenge with the marine Gram negative bacteria, *Vibrio diazotrophicus*, sea urchins respond with an increase in the number of phagocytes expressing *Sp185/333* genes. When single phagocytes were evaluated for *Sp185/333* gene expression, amplicons showed almost identical sequence within single phagocytes suggesting expression from a single gene. The implication is a complex regulatory mechanism may be involved in expression from this gene family.

Cheng Man Lun (George Washington University, USA) described some new aspects of the Sp185/333 protein functions, which share an overall structure of a glycine rich region and a histidine rich region, yet with significant sequence diversity. A recombinant Sp185/333 protein, rSp0032, showed saturable binding to *V. diazotrophicus* and Baker's yeast (but not to *Bacillus* species). rSp0032 also had binding specificity for LPS, β,1-3,glucan, and a non-glycosylated version of flagellin, but not for peptidoglycan. When the glycine rich and histidine rich regions were separated, they showed changes in activities and also bound to the *Bacillus* species. rSp0032 was characterized as hydrophilic and intrinsically disordered but, in the presence of LPS, it transformed from disordered to α helical, and consequently was renamed rSpTransformer. Sp185/333 proteins are present on the surface of some coelomocytes, and preliminary work demonstrated that SpTransformer bound to phosphatidic acid (PA), a small phospholipid with a phosphate head group that is a minor lipid in cell membranes. Similar to responses to LPS, SpTransformer switches from disordered to α helical in the presence of PA. When rSpTransformer was added to liposomes with PA, the outcome was liposome leakage, lysis, budding, fusion and invagination in addition to extraction of PA from liposomes. SpTransformer and perhaps all Sp185/333 proteins may have multitasking activity.

Neutrophil Extracellular Traps (NETs) are composed of nuclear DNA that is released from cells during NETosis. NETs are recognized as an important innate immune strategy because they are embedded with antimicrobial proteins and capture and destroy invading microorganisms. In mammals, NET formation is initiated when cells are stimulated with pathogen associated molecular patterns (PAMPs), including the involvement of protein kinase C

(PKC) in signaling, NADPH oxidase-mediated reactive oxygen species (ROS) production, and chromatin decondensation and ejection of DNA decorated with granules. Andre Van (University of Stirling, UK) described NETs from neutrophils in rainbow trout (*Oncorhynchus mykiss*) after stimulation with LPS, heat inactivated bacteria, phorbol 12-myristate 13-acetate (PMA, a PKC stimulant), or the NADPH catalase inhibitor diphenyleneiodonium chloride (DPI). Results showed that neutrophils were capable of NET generation, that the NETs were composed of DNA, and that NET release was decreased by DPI, suggesting that NET production was dependent on NAPDH oxidase in fish as previously shown in mammals and invertebrates. Valerie Smith (University of St Andrews, Scotland, UK) described NETs in the crab, *Carcinus maenas*, which were released from hemocytes in response to LPS or PMA, could be inhibited by DPI, and were decorated with histone H2A. NETs function as a scaffold to aggregate other hemocytes and act as an important mediator of encapsulation and clearance of microbes from the hemocoel of crabs.

The 13th Congress of ISDCI came to a close after a week of exciting science and reports of new discoveries. Attendees had great fun and went home with new ideas, new collaborators, and new excitement for comparative immunology.

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