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Conference report: The 14th congress of the International Society of Developmental and Comparative Immunology

The 14th Congress of the International Society of Developmental and Comparative Immunology moved back to the United States this past summer. Delegates gathered at the La Fonda on the Plaza in Santa Fe New Mexico June 17th to 21st 2018. Multiple parallel oral sessions, four plenary talks, and two poster sessions brought together immunologists studying diverse model species from around the world. An additional special session focused on origins and evolution of immunity, workshops discussed mentoring, scientific communications and women in STEM, Purnima Bhanot of the National Science Foundation discussed funding opportunities in the IOS: Symbiosis, Defense and Self-recognition program, and society founder and historian Edwin Cooper signed his book. Here we report some highlights communicated during the talks.

1. Plenary sessions

Julie Pfeiffer (University of Texas Southwestern Medical Centre, USA) spoke on the relevance of intestinal microbiota in promoting viral infections. Besides mammalian digestion, the gut bacteria have recently emerged as a key interface in a range of physiological responses. The role of intestinal bacteria in enteric disease development is a particularly active area of investigation, as these bacteria may become feasible therapeutic targets. Accordingly, certain viruses, such as poliovirus and reovirus, take advantage of gut microbiota to establish an infection. Pfeiffer's research team demonstrated that enteric viruses attach to polysaccharides, such as lipopolysaccharides (LPS) or peptidoglycan, found on the bacterial cell surface. The virion binding to bacteria augmented viral stability and receptor binding which in turn enhanced viral replication, pathogenesis, and dissemination. Importantly, mutant viruses with a defect in LPS binding did not efficiently attach to bacteria and had lower infectivity and transmission rates. Interestingly, viruses attached to the bacteria had distinct genomes. The coinfection of genetically diverse virions increases the chances for recombination events that in turn may facilitate infection and disease development. These studies raise important questions regarding the impact of the intestinal microbiome on viral diseases. Controversially, antibiotics may be indirectly antiviral in this scenario. However, as Pfeiffer explained, more research must be performed before jumping to such conclusions as all of the influences of the immune system and enteric viruses and microbiota upon one another remain unclear.

Jules Hoffmann (University of Strasbourg, France) gave an insightful perspective on insect immunity. Although insects do not have adaptive immunity, they are resistant to a myriad of pathogens that in mammals cause severe diseases. Hoffmann's group investigated this phenomenon in the model fruit fly *Drosophila melanogaster*. To fight infection, *Drosophila* accumulated antimicrobial peptides in the haemolymph. Using a variety of genetic and molecular approaches, the group confirmed that these potent defenses involved the products of the Toll gene. Importantly, the mutant flies with deficiency in the Toll gene were highly susceptible to microbial infections. Furthermore, the group established that the Toll gene was necessary for sensing infections and for activating NF-κB signaling in two cascades. Strikingly, these signaling components are evolutionary conserved and many Toll-like receptors (TLR) have been characterized in mammals. Similarly to flies, humans with defects in TLR experience chronic infections. Although some components are different, these antimicrobial systems are a fundamental defense mechanism of complex animals, ranging from insects to humans. The research performed in *Drosophila* innate immunity opened new avenues for preventing and treating human infectious diseases. Importantly, these findings affected other areas of mammalian research. It has more recently become evident that the clinical relevance of TLR may not only be limited to innate immune responses, but also expand to such areas as sleep and behavior. For redefining our understanding of innate immunity and TLR impact on broader biology, Hoffman shared the Nobel Prize in Medicine in 2011.

Xinnian Dong (Duke University, USA) provided a plenary presentation discussing immunity of a group of organisms not well represented at ISDCI, plants. While many molecular mechanisms of animal immunology are conserved, plants have also adapted unique strategies to counteract pathogens. A continuing theme during Dong's talk was the necessary trade-off between plant growth and defense physiology. The decision between the two expenses is orchestrated by numerous transcription factors, such as NPR1. Exogenous application of salicylic acid is sufficient to activate NPR1 and establish systemic acquired resistance (SAR), an inducible broad-spectrum defense mechanism against infection. Salicylic acid was also found to reinforce the circadian clock to temporally regulate immune responses. Plants gate their immune responses towards the morning to anticipate infection while minimizing fitness costs on plant growth, which occurs mainly at night. Much of the recent work in the Dong lab revolves around characterizing an additional transcription factor, TBF1, which controls the growth-to-defense transition in plants. Three open reading frames were identified in the TBF1 transcript. This provides a mechanism to control expression of the TBF1 protein translationally, as was demonstrated by the Dong lab through ribosome footprinting experiments. In sum, plant immunology is tightly regulated both transcriptionally and translationally. Such studies of sophisticated plant immune mechanisms certainly have a home at ISDCI.

Chris Amemiya (University of California Merced, USA) is key in many genome sequencing projects, including the recently completed coelacanth, spotted gar, and lamprey genomes. The sequencing of the lamprey germline genome revealed the occurrence of unheralded programmed genome rearrangement. The germline genome contains several hundred genes that are consistently removed from the genome of somatic cells during early development of lamprey. Elimination of these genes is thought to be related to the maintenance of stem cell identity

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and the silencing of oncogene expression, among many other hypothesized functions. In addition, Amemiya described the rearranging immune antigen receptor genes in lamprey. Rearranging immunoglobulin genes arose between jawless and jawed vertebrates, and are therefore absent in lamprey, a jawless fish. Instead, lamprey utilize the variable lymphocyte receptor (VLR) system to achieve a comparable immune repertoire. While jawless fish lack recombination activating genes characteristic of the vertebrate immunoglobulin system, cytidine deaminase 1 and 2 (CDA1/2) are present in the genome of lamprey. Amemiya described how recombinant lamprey CDA1 is able to function similar to activation induced cytidine deaminase (AID) from jawed vertebrates and convert deoxycytidine bases to deoxyuridine. This work has fundamental impact on our understanding of somatic genomic utilization and the genesis of vertebrate adaptive immunity.

2. Vertebrate innate immunity

Innate immunity is a crucial defense system that rapidly guards an organism from pathogens and contributes to activation of adaptive immunity. Amulya Yaparia (George Washington University, USA) discussed innate defenses in African clawed frogs (*Xenopus laevis*), specifically, the two main subsets of macrophages that respond to viral infections. Data suggest IL-34 induced amphibian macrophages have a robust antiviral response and that this subtype is a functionally differentiated antiviral effector cell type. However, these subsets are not necessarily synonymous with M1 and M2 phenotypes documented in mammalian macrophages. Barbara Katzenback (University of Waterloo, Canada) also used *Xenopus* to explore epithelial viral responses. Due to high permeability, amphibian skin is thought to be a crucial barrier to external pathogens, however functional experiments have been lacking. Using epidermal skin derived cell lines, both pro-inflammatory and anti-viral responses were detected which suggests that frog skin can sense viral pathogens and ellicit anti-viral responses. In addition to amphibian innate defenses, several presentations highlighted novel strategies used by teleost fish. Dustin Lillico (University of Alberta, Canada) investigated multiple pathways and receptors that lead to the development of filipodia-like structures that mediate phagocytosis in channel catfish (*Ictalurus punctatus*) leukocytes. Hiroaki Suetake (Fukui Prefectural University, Japan) investigated circulating basophils in fugu (*Takifugu rubripes*) and found that fish basophils have both antigen-presenting and Ig-dependent qualities. These basophils may be an evolutionary stepping-stone between mammalian basophils and mast cells. Chris Secombes (University of Aberdeen, UK) discussed the expansion of interferons (IFN) in bony fishes, particularly salmonids, and their roles in anti-viral defense. Their analysis of IFNs from cartilaginous, bony and lobe-finned fishes suggest that the ancestral vertebrate IFN likely had a Type II-like five exon/four intron genomic structure and four (as opposed to two) conserved cysteine residues.

Diverse innate cell subsets including the primordial monocyte lineage utilize conserved signaling pathways and effector functions. A novel approach to discern M1 verses M2 activation phenotypes in a teleost model was highlighted by Annelieke Wentzel (Wageningen University, Netherlands). Metabolites were measured as an indication of glycolytic activity (M1) and oxidative phosphorylation (M2). This assay is applicable across a wide range of species used by congress delegates. Bertrand Collet (INRA Université Paris-Saclay, France) discussed an adaptation of the CRISPR/Cas9 system for use in salmonids. The importance of STAT2 (signal transducer and activator of transcription 2), a potent regulator of the IFN response in immune cells, was elucidated in teleosts. Null mutations for *stat2* were successfully generated and verified, broadening immune signaling use of the CRISPR system in lower vertebrate species. The genome duplication events that occurred in fish left multiple paralogs of heterologous proteins expressed in these species. Tiehui Wang (University of Aberdeen, UK) described fish Interleukin-2 (IL-2) isoforms, IL-2A and IL-2B, and their conserved roles in T cell development, proliferation and function in trout. In *Xenopus laevis*, IL-8 evolution exhibits bi-modal regulation of immunity. Leon Grayfer (George Washington University, USA), described two IL-8 isoforms with unique expression patterns; IL-8a displaying characteristic CXC chemokine features including chemotactic activity, and IL-8b that lacks CXC active motifs similar to many teleost inflammatory chemokines and exhibits immunosuppressive function in tadpoles. In common carp, G-CSF (granulocyte colony-stimulating factor) paralogs were described by Fumihiko Katakura (Nihon University, Japan). Two isoforms are constitutively expressed at basal levels in many tissues and all four are upregulated upon LPS stimulation in myeloid cells. IFN-y was examined in orange-spotted grouper by Hong-ye Ong (National Cheng Kung University, Taiwan). In addition to IFN-y, orange-spotted grouper also produces IFN-yrel, where IFN-y displays more similarity to the mammalian ortholog. IFN-y was shown to be expressed in gill and fin primarily in response to viral stimuli and IFN-yrel was shown to be expressed foremost in the eye in response to bacterial stimuli. Tiehui Wang (University of Aberdeen, UK) reported two divergent IL-2 paralogs in trout and demonstrated the capacity of recombinant protein from these paralogues to induce the expression of Th1 pathway cytokines and promote the growth of peripheral blood leucocytes. These paralogs are differentially expressed *in vivo*, but both appear to be integral regulators of the Th1 and Th2 pathways in fish. In a similar system of investigation, Jingqun Ao (Third Institute of Oceanography, China) identified two IL-4/13 homologues from large yellow croaker and showed that they reduce ROS and nitrogen oxide production, decrease proinflammatory cytokine expression, and inhibit phagocytic activity in macrophages.

3. Mucosal immunity

The mucosal immunity session explored the intricacies of host-pathogen interactions in a number of teleost species, lungfish, and an invertebrate deuterostome (urchin). Fumio Takizawa (University of Pennsylvania, USA) began the session with a unique model for depleting IgT^+ B cells in rainbow trout, which highlighted the importance of mucosal antibodies in regulating both commensal and pathogenic microbial populations. Section chair Elisa Casadei (University of New Mexico, USA) next discussed the role of olfactory signaling, which was shown to be heavily manipulated by microbial populations, specifically increasing expression of the RE1-Silencing Transcription Factor (REST) in both zebrafish and mice. Sylvia Brugman (Wageningen University, Netherlands) shared her data centered on the role of T cells in preventing dysbiosis orchestrating responses via neutrophil recruitment in an induced colitis model, and through adoptive transfer of T cells into RAG deficient fish. Stepping out of the water, Ryan Heimroth (University of New Mexico, USA) used the lungfish model to study the effects of terrestrialization. His study concluded that, in addition to an increased expression of immune related genes in terrestrialized animals, antibody expression in the Dipnoid skin shifts the constitutively skin-localized IgM1 to a terrestrially-exclusive phenotype of both IgM1 and IgM2 in the integument. Next Katherine Buckley (Carnegie Mellon Institute, USA) working with the larval stage of the purple sea urchin, demonstrated that IL17 has played an integral role in mucosal

immunity throughout deuterostome evolution. Geert Wiegertjes' (Wageningen University, Netherlands) work focused on the organization of interbrachial lymphoid tissue, finding compartmentalized lymphocyte populations of B and T cells. Wrapping up the session was Giuseppe Scapigliati (Tuscia University, Italy) with the characterization of CD3ε$^+$ T cells in sea bass with an emphasis on their role in not only the mucosal, but systemic pathogen defense.

Emily Flowers (University of Maryland, USA) has been studying the ~80 FcR-like genes of the frog *Xenopus laevis*, and found orthology between what in frog was called polymeric Ig receptor 2 and mammalian FcαμR. This gene is expressed on frog antigen presenting cells in spleen and binds IgM and IgX (precursor of mammalian IgA) but not monomeric IgY (precursor of IgG and IgE). Bethany Fehrenkamp (University of New Mexico, USA) studied the immunologically immature neonates of marsupials who rely on milk factors for immunity. She found an increase in IgM and IgA expression in mammary gland across lactation as well as accompanying FcαR and FcRN there, suggesting transport into the milk from maternal circulation. Yu-Hsien Chou (National Cheng Kung University, Taiwan) found that IgT in the orange spotted grouper is upregulated upon immune stimulation and is upregulated in larval gut upon treatment with nervous necrosis virus. It has been difficult to find organized lymphoid structures in teleost fish, but Oriol Sunyer (University of Pennsylvania, USA) has found evidence for such aggregations in rainbow trout. A panel of fish monoclonal antibodies his group developed localized to sites of CD4 T cell activation and B cell proliferation in the spleen, gill and gut in response to pathogen. Chytrid fungus have contributed to global amphibian declines, and have developed novel counter-defenses to frog adaptive immune responses. Louise Rollins-Smith (Vanderbilt University, USA) identified and functionally characterized two small immunomodulatory molecules (kynurenine and methylthioadenosine) that inhibit lymphocyte proliferation. Keiichiro Koiwai (Tokyo University of Marine Science and Technology) employed hemocyte populations from various tissues of the kuruma shrimp and transcriptomics to find that infiltration into tissues may cause functional differentiation of sessile hemocytes. An immune role for olfactory sensory neurons was found by Ali Sepahi (University of New Mexico, USA) studying the olfactory organ crypts in trout and zebrafish. Shown by experiments with rhabdovirus, olfactory sensory neurons and their surrounding tissue appear to be an important first line defense against mucosal pathogens in the nares.

Novel function of IgT$^+$ B cells was reported by Yasuhiro Shibasaki (University of Pennsylvania, USA). Comparative transcriptome analysis and RT-PCR analysis between IgT$^+$ and IgM$^+$ B cells showed that perforin, a cytolytic molecule not traditionally associated with B cells, was highly expressed in IgT$^+$ B cells. Perforin isoforms were not only expressed at the transcript level, but also at the protein level. They also reported that IgT$^+$ B cells have NK-like cytotoxic capacity. Ryan Brown (University of New Mexico, USA) described challenge approaches to reveal the relationship between disease resistance and microbiome analysis in trout. Swim bladder and lung are thought to have evolved from the primitive lungs of a common ancestor, and Zhen Xu (Huazhong Agriculture University, China) reported previously unrecognized mucosal immune responses in swim bladder. Virus specific IgT responses were locally produced in the swim bladder mucosal associated lymphoid tissue (SBALT). Jun-ichi Hikima (University of Miyazaki, Japan) reported the role of IL-17 in the intestine through detailed analysis of knock-out medaka. IL-17A/F1 KO fish showed attenuation of immune response and metabolism in the gut. Antigen sampling mechanisms on the gill surface were reported by Goshi Kato (Tokyo University of Marine Science and Technology, Japan). His group identified two different phenotypes of antigen sampling cells, monocyte/macrophage/dendritic cell-like and M cell-like, which suggests the presence of a novel antigen uptake and presentation mechanism in mucosal immunity in trout.

4. Parasite immunology

Lijun Lu (University of New Mexico, USA) reported on the immune responses of the trematode host snail, *Biomphalaria glabrata*. A challenge experiment with helminths and subsequent gene expression analysis showed different patterns indicating distinct host immune responses against these parasites. Two talks about myxozoa and teleost fish interaction were presented by Tomás Korytář (Ceske Budejovice, Czech Republic) and Laura M. Taggart-Murphy (Oregon State University, USA). Korytář reported on immune responses of carp against *Sphaerospora molnari* and their infection dynamics. *S. molnari* proliferates in the host vascular system prior to spore formation in the epithelia of gill and skin. Taggart-Murphy showed differential expression analysis of cytokines that distinct genotypes of *Ceratonova shasta* induces for a harmful inflammatory response. Jonathan Schultz (University of New Mexico, USA) discussed a comparative transcriptomic analysis among gastropods. The results indicate *Physella acuta* has unique immune competence that enable the species to be a globally invasive freshwater snail. Eric Kenney (George Washington University, USA) described the immunosuppressive function of excreted-secreted products of entomopathogenic nematodes on *Drosophila*. The results provide strategies for biocontrol of agricultural pests. Judith Humphries (Lawrence University, USA) reported how NF-κB is conserved in selected TLR pathways in *B. glabrata*. Innate-like T cell function was reported by Maureen Banach (University of Rochester, USA) with RNAi showing that the MHC I-like gene, XNC10, is required for development and function of innate-like T cells in *Xenopus*.

Exploring host-parasite interactions between the host model system *B. glabrata* and parasite *Schistosoma mansoni*, Hongyu Li (Qinzhou University, China) explored the family of immune genes known as FREPs (fibrinogen-related proteins) in relation to parasite infection and defense. His group found that FREP2 interacts with TEP (thioester-containing protein) and galectin during infection. FREP3 is capable of independent binding to *S. mansoni* while FREP2 is not. This binding correlated with an increase in opsonization and encapsulation of the sporocysts. Jacob Hambrook (University of Alberta, Canada) explored the success of infection from the parasite's perspective. Looking at the expression of the zinc-dependent metalloprotease *SmLeish,* they showed that it is expressed in two key life stages of *S. mansoni* infection: within miracidia and upon cercaria release. Shifting to vertebrates, Tea Sung Jung (Gyeongsang National University, South Korea) presented on the structure of the hagfish VLRB. The C-terminal end of VLRB in hagfish showed super-hydrophobicity which drives the formation of a multimeric structure with the VLRB pattern recognition receptors facing outward. This makes its structure different from the lamprey VLRs. This might be indicative of key immunological differences between lampreys and hagfish. Doaa Waly (University of Alberta, Canada) presented a talk on antibody affinity maturation in fish and the genesis of the germinal centre reaction in vertebrate evolution. Further up the phylogenetic Yang Ding (University of Pennsylvania, USA) presented work in which they showed that mouse splenic plasma cells do not only function in anti-body secretion but are also capable of phagocytosis. Challenging mice with LPS leads to an increase in phagocytic plasma cells (PhPCs) which could suggest an early immune response function in plasma cells.

5. Vaccines

As the aquaculture industry continues to grow, there is an increasing need for vaccine development to protect economically important species against harmful pathogens. Once such disease, Salmon Anemia Virus (SAV), is a widespread disease among salmonids. Noelia Nunez-Ortiz (Institute of Marine Research, Norway) presented on the adaptive immune response of Atlantic salmon to SAV3 vaccination through intramuscular injection versus bath immersion. Differences were found in cellular and humoral gene expression between fish treated with the two methods as well as an increase in white blood cells in the peritoneal cavity. Additionally, RNA-seq analysis identified that triploid fish had a higher up-regulation of antiviral genes and pattern recognition receptors compared to diploid. Ingvill Jenson (University of Tromso, Norway) investigated the comprehensive immune response after intraperitoneal injection with SAV and monitored B cell response for up to 9 weeks later. Infection peaked two weeks post challenge, when an increased leukocyte influx and rising number of IgM secreting cells in the peritoneal cavity was observed. Meanwhile there was a decreased number of leukocytes and IgM secreting cells in the peritoneal cavity. Over a longer term there was an increase in leukocytes and secreting cells in the peritoneal cavity but only minor changes in the systematic lymphoid tissues. This identified the peritoneal cavity as a key immune site.

Ahmed Attaya (University of Aberdeen, UK) also looked at the immune response to vaccines in salmonids in his research on the development of novel nanoparticle based oral vaccines. Oral vaccines are one of the best methods for vaccinating aquaculture species but there are still problems with delivery due to the lack of efficient encapsulation that will protect the antigen from gastric degradation but still allow for uptake in the intestines. To solve this problem, they evaluated a novel silicon nanoparticle-based delivery system known as ProSilic to deliver *Aeromonas salmonicida* antigens. This system is both biocompatible and biodegradable. Various formulations were tested for their stimulatory impact on different cell types and stability to acidic media. Yehfang Hu (University of Aberdeen, UK) presented on the role of interferon-y in the rainbow trout immune response. IFN-y orthologs have been discovered in fish species which suggests that the Th1 type immune response is present in early vertebrates. In this study several monoclonal antibodies were validated against different peptide immunogens and all reacted specifically in both ELISA and western blots. Marc Nicolas Faber (University of Aberdeen, UK) also presented on rainbow trout, characterizing biomarkers and testing novel treatments of proliferative kidney disease in rainbow trout. The study used RNA-seq analysis to analyze early fish host response following parasite invasion of the gills. The parasite transcriptome assemblies were useful to uncover parasite antigens for vaccines studies.

Invertebrate species are also potential candidates for vaccine development. Thu Nguyen (University of Tasmania, Australia) presented on how secondary exposure of the tropical rock lobster *Panuliris ornatus* enhances antibacterial activity. This memory response lacks specificity but increases signaling pathways, cellular mechanisms, and expression of specific receptors in the lobster upon repeated challenge.

6. Invertebrate innate immunity

A variety of invertebrate model systems offer insight on the evolution of innate immunity. The innate immune system is already known to play important roles in the nervous system of vertebrates, including brain development and tissue homeostasis. Chadanat Noonin (Uppsala University, Sweden) presented evidence that the immune system participates in neural repair in freshwater crayfish (*Pacifastacus leniusculus*). Neural damage was induced by severing crayfish antenna. This resulted in an increase in circulating granulocytes and hemocytes, as well as an increase in astakine 1 levels in the brain, a hematopoietic cytokine. Granular hemocytes were recruited to the brain in response to the injury, suggesting an intimate relationship between innate immune cells and repair of the nervous system in invertebrates. Irene Söderhäll (Uppsala University, Sweden) also presented on hematopoiesis in the crayfish model. Quantitative global proteomics have proved a useful tool for discriminating blood cell lineages and neuronal precursor cells, both of which arise from shared stem cells in the hematopoietic tissue.

Characterization of hematopoiesis in invertebrates extends further to the tunicate (*Botryllus schlosseri*) system, as reported by Benyamin Rosental (Stanford University, USA). Insightful use of flow cytometry and CyTOF allowed for the sorting and identification of distinct immune cell populations in this invertebrate. Whole-transcriptome sequencing of these cell populations identified hematopoietic stem cells (HSCs) and immune effector cells. Functional assays helped confirm the presence of a cytotoxic cell population that mediates self/non-self-recognition reactions, as well as a potential hematopoietic stem cell niche. These results not only demonstrate the early origins of hematopoietic stem cells and the myeloid lineage in a basal organism, but they highlight the usefulness of flow cytometry and transcriptomics to develop unbiased approaches for identifying immune cell populations in less well studied systems. In addition to these flow cytometry techniques, Megan Barela Hudgell (George Washington University, USA) reported on a new method for lipofection of nucleic acids into larval cells of the purple sea urchin (*Strongylocentrotus purpuratus*) to generate transgenic organisms. Cell mortality was reduced at high salinity and low temperature conditions.

Another useful invertebrate phylum for studying innate immunity is Cnidaria. Juris Grasis (University of California, Merced, USA) discussed the interactions of the virome and the holobiont in *Hydra*. This simple multicellular organism exhibits complex interactions between host immunity and species-specific viral communities. The *Hydra* respond strongly to the presence of foreign virus infection, but are tolerant to exposure to previously established viruses, a system believed to be mediated by the presence or absence of stimulatory bacterial pathogen associated molecular patterns (PAMPs). Matthew L. Nicotra (University of Pittsburgh, USA) presented on allorecognition in another cnidarian model, *Hydractinia symbiolongicarpus*. *Hydractinia* alloresponses are regulated by genes in the allorecognition complex (ARC), from which multiple new genes were reported with high levels of allelic polymorphism. Binding of ARC-encoded gene products on organisms sharing haplotypes is believed to mediate self/non-self-discrimination. Future studies of the evolution of these genes will provide a greater understanding of how many other immune genes exhibiting high diversity have been generated.

Returning to crustaceans, Elisabeth Dyrynda (Heriot Watt University, UK) and Valerie Smith (University of St. Andrews, UK) discussed the protein carcinin, a crustin antimicrobial peptide found in the crab *Carcinus maenas*. This protein is produced by semi-granular and granular cells of the crab haemolymph, which then deposit the protein in tissues such as the gill, heart, and gut. It is believed to provide protection against microbial infection and to facilitate wound healing. Valerie Smith's research demonstrated that carcinin can bind bacteria and kill them via pore formation. It also provides opsonic activity in the presence of phagocytic cells. The putative receptor for carcinin on the surface of phagocytic cells remains to be discovered, but

these results demonstrate the diversity of molecular mechanisms invertebrates have evolved to mediate immune defense. Another study with a direct impact on mud crab production was presented by Xiaowan Ma (Xiamen University, China). He described the arsenal of immune associated genes expressed in twelve early developmental stages and is working to develop a gene expression profile of high mortality in early stages of mud crab aquaculture.

Kingkamon Junkunio (Uppsala University, Sweden) discussed hematopoiesis in the crayfish model. The role of PDGF/VEGF related receptor in signaling pathways influencing cell morphology during hematopoiesis was characterized. Recent advances in knowledge of immune genes in bivalves have led to significant progression in understanding of innate immunity in non-model organisms. Linsheng Song (Dalian Ocean University, China) elaborated on important immune defense mechanisms in bivalves as demonstrated by a network generated from 300 genes cloned from oysters and scallops. Presence of several pathogen recognition receptors and important signaling pathways were reported to be present in bivalves, with immune priming both within and across generations. In a gastropod mollusk the role of thioester containing proteins (TEPs) in snail defense mechanisms against schistosomes was reported by Deblina Misra (New Mexico State University, USA). Her study showed presence of five TEPs that belong to three known phylogenetic groups. A cell line exposed to *Schistosoma mansoni* and other microbial products was used to study transcription regulation of TEPs. Michael Tassia (Auburn University, USA) discussed the evolution of TLR signaling pathways in deuterostomes by studying 37 deuterostome genomic and transcriptomic datasets. Using bioinformatics tools, evidence for the presence of the canonical MyD88-dependant TLR signaling pathway in the most recent common ancestor of deuterostomes was presented.

7. Immune responses to bacteria

Recognition and clearance of bacterial pathogens is central to immunity and unique interactions have evolved across diverse animal species. Kimberly Veenstra (University of Aberdeen, UK) challenged the dogma of what we consider immune tissue in fish. In rainbow trout, *Oncorhynchus mykiss,* a transcriptional approach was used to analyze visceral adipose tissue (omentum) for immune response after vaccination. Considering fish vaccination routes, adipose tissue may play a vital role in the immune response and should be characterized as an active immune site in fish. Estefania Muñoz-Atienza (CISA-INIA, Spain) presented work on the rainbow trout chemokine, CK11. The CC chemokine is not chemotactic yet exhibits potent antimicrobial activity. This is the first fish chemokine described with antimicrobial activity which supports the evolutionary relationship between chemokines and antimicrobial peptides. Kun Hyoe Rhoo (University of Rochester, USA) works with the amphibian model, *Xenopus laevis*, and characterized the macrophage response to *Mycobacterium marinum* infection. Interaction of specific invariant (i) T cell subsets with XNC4 was found to be indispensable for host protection to the mycobacterial infection. Hidehiro Kondo (Tokyo University of Marine Science and Technology, Japan) described four distinct Fc receptor-like proteins in Japanese Flounder, *Paralichthys olivaceous*. All four bind flounder IgM and two bind bacterial cells highlighting their importance in immune responses targeting pathogenic bacteria.

Other important aspects of pathogen response are genetic background and breeding fitness. Shawna Semple (University of Waterloo, Canada) studied immune differences between eight outbred chinook salmon populations from geographically distinct locations. Phenotypic and genetic information about natural immunity against pathogens is vital to develop breeding strategies for fitter, more productive fish. Jules Petit (Wageningen University, Netherlands) argued that due to the evolutionary position of teleosts as early vertebrates with fully developed immune systems, fish cells possess capacity for trained immunity. The concept, exhibiting a non-specific, heightened second response to stimuli, has recently been described in humans and mice. An *in vitro* screening model was established using macrophage from common carp, where a heightened response in trained cells was observed validating the model to screen for trained immunity in teleosts.

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor which has roles in regulating immunity, stem cell maintenance, and cellular differentiation. Jiannong Xu (New Mexico State University, USA) described the role of AhR in the negative immune regulation in two insect models, mosquito *Anopheles gambiae* and fruit fly *Drosophila melanogaster*. In the mosquito model, it was found that mosquitos were more sensitive to the hemocoel infection with bacterium *Serratia* when AhR was activated by feeding agonist kynurenine, showing a higher mortality than the control group. However, the mosquitoes were more resistant to the bacterial infection when AhR was inhibited by feeding antagonists, CH233191 and StemRegenin. RNA interference of AhR gene further support these results with a higher survival rate. In the fruit fly model, the AhR gene is encoded by gene *spineless*, *ss*. Two *ss* loss of function mutant lines, ss^1 and ss^a, showed a higher survival than the wild-type flies when infected by pathogen Bacterium *Providencia*. Consistently, the survival of the infected wild type flies was also increased when they were treated with antagonist CH233191. These imply the role of AhR in the negative modulation of insect immunity. Ramya Kumar (National Cheng Kung University, Taiwan) presented on the pathogenesis of *Vibrio parahaemolyticus* in causing acute hepatopaancreatic necrosis disease in the white shrimp *Litopanaeus vannamei*. The team found a crucial role for bile acids and their transporters in the stomachs of affected shrimp.

Then Tejashree Modak (University of Rhode Island, USA) introduced a new strategy for use of probiotics to control disease in oyster hatcheries. The eastern oyster *Crassostrea virginica* larvae were exposed to probiotics daily for 16 days. Transcriptomes showed the different gene expression profiles involved in immune signaling pathways, apoptosis and metabolic pathways between probiotics treatment and control. Challenge experiment with *Vibrio coralliilyticus* showed that pretreatment of larval oysters with probiotics resulted in an increased survival than the controls. Finally, Sven Ostermann (Friedrich-Loeffler Institute, Germany) compared two models for characterizing the innate immune response of *Oncorhynchus Mykiss* after stimulation with inactivated *Aeromonas salmonicida* by oral and intra peritoneal injection. By flow cytometry, they analyzed the distribution, recruitment and migration of several specific myeloid cell population after infection. Moreover, they also characterized these populations using specific makers of macrophages (GCSFR, CD68), NK-T cells (NCCRP1) and dendritic cells (CD83, CD209), and the expressed cytokines (IL-1b, IL-6, IL-8, IL-10, TNF and IFNg). The goal is to establish an oral model for stimulation with bacterial antigen and address the mucosal innate immune response in teleost fish.

8. T cells, TCR and MHC

Veterinary medicine is often where clinical methods are developed that can later be translated to human medicine. In particular, the extensive breeding of dogs has provided more homogeneous populations (21% homozygous), which simplify challenges in MHC alloreactivity.

Jiro Miyamae (Nihon University, Japan) utilized this more homogeneous population structure to test the use of healthy MHC homozygous canine stem cells for regenerative medicine in MHC heterozygous recipients. While partial match recipients were more alloreactive than full match recipients, they were less alloreactive than full mismatch recipients. Julie Old (Western Sydney University, Australia) aimed to characterize MHC diversity in wombats to see if certain haplotypes were more or less susceptible to sarcoptic mange. This disease is debilitating for this species and can cause mortality through secondary infections. However, sample collection from individuals is limited due to their furtive habits and their endangered status. Therefore, she had to utilize fecal samples and take opportunity of roadkill. The wombat also does not have an assembled genome, so she had to rely on MHC primers from other marsupial species. Even with the limitations, she was able to identify 42 unique alleles at one MHC locus from 90 samples. Yogesh Khandokar (Monash University, Australia) presented his discovery of ligands for the chicken non-classical MHC molecule, YF1*7.1. This molecule was phylogenetically more related to MR1 than the CD1 group, however the ligand is not the same at human MR1. His work underscored how biochemical studies are necessary to understand the functions of these molecules, as sequence homology does not equate functional homology.

Dustin Wcisel (North Carolina State University, USA) sought to find a possible homolog of the bony fish novel immunoglobulin-like transcripts (NILTs) in human. He used the spotted gar as an intermediate between bony fish and human since they did not have the genome duplications of the bony fish. He was able to identify connections between the zebrafish NILTs and the spotted gar PIGR, however the human orthologs remains elusive. The teleost-specific IgT is a mucosal antibody isotype that is different from the human IgA. It has been well studied in the mucosal surfaces in teleosts, such as their skin and gills. However, Carolina Tafalla (Centro de Investigación en Sanidad Animal, Spain) discovered a novel function of this antibody isotype. While looking at how *Tetracapsuloides bryosalmonae* caused B cell dysfunction in proliferative kidney disease, she discovered that the IgT isotype was the prevalent antibody in the immune response in the kidney. Therefore, this antibody isotype may not be restricted to mucosal immune responses as previously thought.

Based on numerous studies conducted in mice and humans on the vertebrate adaptive immune system, we presume T and B cell loci are distinct from one another. However, current research in non-model organisms forces us to reexamine the distinctness of B and T cell lineages. Thad Deiss (Texas A&M University, USA) presented examples of divergent receptor loci in nurse shark (*Ginglymostoma cirratum*) with shared components of both T and B cells. The shark TCR αδ locus contains nested IgHV segments that rearrange with the TCRδ constant region, creating chimeric IgHV-TCRδ rearrangements. Breanna Breaux (Texas A&M University, USA) also discovered an IgVH-TCRδ pseudogene in the Florida manatee (*Trichechus manatus latirostris*), the first known occurrence of this gene in a eutherian mammal. Further, the Florida manatee TCR αδ locus exhibits a high degree of synteny to the human TCR αδ locus. By comparing the TCR loci between groups of mammals, Breaux identified an additional Ig-like TCRδ-V segment within the Florida manatee locus that is highly syntenic to an Ig-like TCRδ-V segment in other mammals previously misidentified as TCRδ-V, underlying the need for a comparative approach to studying immunology.

Matthieu Paiola (Normandy University, France) described how estrogen regulates T cell differentiation and immune tolerance in European sea bass (*Dicentrarchus labrax*). As estrogen levels increase during pregnancy, there is a corresponding increase in thymic atrophy, inhibiting αβ T cell differentiation. However, γδ T cells increase, suggesting that estrogen may promote γδ T cell proliferation. Jacques Robert (University of Rochester, USA) reported a novel innate-like T cell (iT) subset (Vα45-Jα1.14) in *Xenopus laevis* that requires a distinct MHC I-like molecule for its development and critical function in host defense against mycobacteria. Similar to the CD1d-restricted iNKT cells observed in mammals, Robert's results suggest that immune surveillance using MHC-like iT cells is more widespread within jawed vertebrates than previously thought. Karen Tracy (University of California, Davis, USA) analyzed T and B cell repertoires of two genetically distinct chicken lines with inherent differences in susceptibility to Newcastle disease virus. Differences in baseline TCR repertoires may influence cellular immunity. Tracy showed that the more resistant Fayoumi birds have a greater diversity of TCR clonotypes and use different V and J segments pre-infection than the less resistant Leghorn birds. After infection, public TCR clonotypes frequency increases in Leghorns but decreases in Fayoumis, suggesting a negative impact on viral resistance under heat stress. Further, a larger increase in IgY clonality in Fayoumis after treatment may contribute to a higher viral resistance.

9. B cell and Ig

In mammals, two cytokines of the tumor necrosis factor ligand super family (TNFSF) influence B cell maturation and survival: B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL). Several researchers reported on the significance of these cytokines in teleost and cartilaginous fishes. Irene Soleto (Animal Health Research Centre, Spain) reported on the influence of an APRIL ortholog in rainbow trout (*Oncorhynchus mykiss*) on splenic B cells. APRIL was found to have different effects on IgM^+ versus IgM^- B cells. Whereas APRIL induced significant proliferation in IgM^+ B cells, IgM^- B cells were not found to respond to the cytokine. APRIL was also found to enhance IgM^+ B cell processing of antigen, and to increase levels of surface IgM and IgM secretion. Michelle Pañaranda (University of Tromsø, Norway) also reported on the role of APRIL in teleost fish. Profiling of APRIL transcript expression from salmon solid tissues and leukocytes revealed high expression in head kidney macrophage-like cells and B cells. Treatment of head kidney B cells with recombinant APRIL promoted IgM^+ B cell survival, but not proliferation. APRIL was also found to promote numbers of antibody secreting cells.

Further investigations into B cell responses in bony fish by Aitor Granja (Animal Health Research Centre, Spain) focused on the role of CD40 ligand (CD40L), which engages CD40 expressed on T cells to provide co-stimulation during T cell-dependent immune responses. In an examination of rainbow trout tissues, CD40L was found to be expressed by T cells and dendritic cells in lymphoid tissues, as expected. Treatment of trout B cells with recombinant CD40L stimulated proliferation and upregulation of IRF4 and IRF8 transcription factors, as well as increased MHC class II and antigen processing. CD40L was found to influence rainbow trout B cells under various antigen conditions, including treatment with LPS. Much like in mammals, CD40L promotes B cell responses in teleost fish.

Returning to the role of TNFSF in vertebrate immunity evolution, Helen Dooley (University of Maryland, USA) presented on the discovery of APRIL and BAFF orthologs, in addition to their receptors BAFF-R, BCMA, and TACI, in cartilaginous fish, the lowest vertebrate group with B cell mediated humoral immunity. Evidence derived from a small-spotted catshark (*Scyliorhinus canicula*) transcriptome indicated the presence of a third TNFSF cytokine, BALM, previously thought to be

bony-fish specific. Phylogenetic evidence suggests APRIL was the founding member of this cytokine family, and that three cytokines, rather than the two found in mammals, were present at the advent of the immunoglobulin-based immune system. How these three cytokines influence B cell development and survival in cartilaginous fish remains to be determined.

Multiple talks highlighted the importance of B cell repertoire development in teleost fish. Pierre Boudinot (Université Paris-Saclay, France) discussed the role of IgM and IgT repertoires in rainbow trout vaccination and memory responses. Naïve IgM and IgT repertoires were found to have different characteristics: IgM clonotypes were frequent and different among individuals, while IgT clonotypes were less abundant and shared among individuals. After immunization, a VH5JH5 rearrangement in the IgM repertoire was found to dominate the immune response across all immunized individual fish, differing in sequence by only one to two amino acids. The B cell memory response persisted several months after virus clearance. The bias of the initially diverse IgM repertoire towards distinct public clonotypes suggests that maturation of the B cell population during an immune response and not the recombination machinery selects for this VH5 rearrangement. This indicates that specific Ig rearrangements or CDR3 sequences present in a population may identify individuals predisposed for successful vaccination. Additionally, Susan Magadán (University of New Mexico, USA) reported on differences in the rainbow trout Ig repertoire in spleen versus nasopharynx associated lymphoid tissue (NALT), located in the olfactory organ. Differences in pre- and post-vaccination state, as well as differences between systemic and intranasal vaccination, suggest that greater diversity exists in the Ig repertoire of cells responding in the spleen than the NALT. Differential V region usage revealed by deep sequencing may indicate that a limited diversity of clones seeds the NALT and dominates the nasal immune response. Together, these data reveal how to better design and apply vaccines in populations of fish.

Finally, Andrew Collins (University of New South Wales, Australia) discussed the recent creation of the Inferred Allele Review Committee (IARC), dedicated to standardizing the reporting of antibody genes across species. The goal of this project is to establish a streamlined pipeline for discovering, annotating, and independently confirming germline immunoglobulin genes in multiple species to provide researchers with a tool for both referencing known genes and comparatively assessing the V, D, and J building blocks of recombination.

10. Immune responses to virus

A variety of organisms from water fowl to shrimp can be used as a model for viral infection. Katherine Magor (University of Alberta, Canada) presented on influenza detection and interferon stimulated genes in White Perkin ducks, a natural host for influenza A virus. The study identified intracellular sensor RIG-I in ducks and showed that it is upregulated and involved in innate immune response. An analysis of genes turned on downstream of RIG-I in low and high pathogenic avian influenza strains found that interferon stimulated genes respond to highly pathogenic virus in the lungs but barely to low pathogenic strains which replicate in the intestine. The magnitude of response in ducks to related highly pathogenic strains was found to correlate with virulence. Takahiro Nagasawa (Kyushu University, Japan) studied the ability of thrombocytes in the common carp (*Cyprinus carpio*) to produce type I IFN in response to synthetic viral nucleic acid mimicries. Thrombocytes and other peripheral blood leukocytes (PBL) were incubated with a variety of analogs for toll like receptors (TLR) such as poly:IC, R-848, and CpG motif oligodeoxynucleotides. qPCR analysis revealed that compared to PBL, the thrombocytes had produced a larger amount of type I-IFN. R-848, an antagonist of TLR7/8 (ssRNA), stimulation drastically increased expression of interferon-regulatory factor 7 (IRF7) in thrombocytes suggesting that the IRF7-signal pathway was used to produce IFN.

Aurora Kraus (University of New Mexico, USA) presented on nasal epithelium response in zebrafish to intranasal delivery of a live attenuated IHNV vaccine. The olfactory epithelium has olfactory sensory neurons which can act as an immune receptor. Trout can smell viruses and bacteria, and this can cause behavioral changes. They found an upregulation of immune genes in the nasal epithelium immediately after vaccination as well as a widening of the lamina propria which indicates inflammation. This was also accompanied by decreased locomotor activity in the first 15 minutes which suggests the olfactory sensory neurons recognize IHNV and send the information to the CNS, changing behavior of the fish. Kelsey Abernathy (University of Maryland, USA) presented on IHNV in zebrafish, looking at the role of recombinant zebrafish tandem repeat galectin 9 (DrGal9-L1) in mediating viral adhesion and infection. Galectins are known to function in the innate immune response as pattern recognition receptors (PRR). DrGal9-L1 interacts with the glycoprotein of IHNV to promote adhesion to the fish epithelial cell surface. Current research is using biochemical and glycomic analysis to characterize the binding activity of Drgal9-L1 C— and N-terminal carbohydrate recognition domains (CRD) to determine if there is any preference for the viral glycoprotein verses the epithelial cell surface.

Szu-Hsuan Mao (National Cheng Kung University, Taiwan) presented on the role of the orange spotted grouper autophagy marker, LC3, in the immune response. Autophagy is a process involved in both the innate and adaptive immune response. The microtubule-associated protein light chain 3 (LC3) is a constituent of the autophagosome and is used as a marker for autophagy. When exposed to immune stimulating agents such as poly I:C and LPS as well as Nervous Necrosis Virus (NNV) the expression of the orange-spotted grouper LC3 gene (*osgLC3*) increased in the brain, eye, and head kidney. Additionally, high temperature exposure effected *osgLC3* expression more compared to low temperature exposure. Meanwhile, Yi-Ting Zeng (Biotechnology & Bioindustry Sciences, Taiwan) presented on how RAS mediates White Spot Syndrome (WSSV) induced Warburg effect for viral replication in shrimp (*Litopenaeus vannamei*). The Warburg effect causes cells to increase in glucose consumption and plasma lactate concentrations at the replication stage. It is unknown what pathway WSSV uses to regulate the Warburg effect. WSSV infection caused an increase in gene expression of RAS1/2 as well as activation of PI3K/Akt and RAS/ERK pathways. RAS was shown to be crucial in triggering WSSV induced Warburg effect as suppressing RAS with Salirasib or silencing it with dsRNA resulted in decrease of WSSV genomic copies.

Bats harbor many viruses asymptomatically that cause extreme virulence in humans. Tom Kepler (Boston University, USA) used the genome of the Egyptian fruit bat to identify expansions of natural killer receptors, MHC genes and interferons. They expressed some of the novel omega family interferons and showed them to be able to inhibit Marburg virus infection in bat kidney cells.

11. Ecoimmunology and stress responses

There is a tremendous amount of variation in immune defense strategies and environmental immunology, or ecoimmunology, provides insights into potential trade-offs between immunocompetence

and other life-history traits. George Brusch (Arizona State University, USA) used a combination of temperature and chemical treatments on Western diamond-backed rattlesnake (*Crotalus atrox*) plasma samples, and found that complement and other small antimicrobial peptides (AMPs) are likely upregulated during dehydration. Stanislava Chtarbanova (University of Alabama, USA) used the fruit fly (*Drosophila melanogaster*) system to examine aging-related chronic inflammation (inflammaging). Results showed that NF-kB transcription and subsequent upregulation of AMPs increases with age and is correlated with neurodegeneration. Furthermore, experimental downregulation of AMPs leads to lifespan extension and healthy aging.

Two presentations in the session described work in mollusks. Coen Adema (University of New Mexico, USA) described next-generation sequencing expression profiling of *Lymnaea stagnalis* in response to different environmental stressors or immune stimulants. Marco Gerdol (University of Trieste, Italy) presented several examples of non-random distribution of genetic variation in lophotrochozoan genomes such as lectins and antimicrobial peptides, thought to be important in the defense of bivalves.

Debora Torrealba (University of Alberta, Canada) explored behavioral thermoregulation to induce fever in goldfish (*Carassius auratus*). After a cutaneous infection, fish were exposed to either a dynamic or static temperature gradient, and fish in the dynamic treatment had functionally enhanced immune responses. Daniel Barreda (University of Alberta, Canada) also explored behavioral febrile responses in goldfish. Using a novel thermal gradient, leukocyte recruitment and pathogen killing abilities were tracked. Changes in acute inflammatory responses were highly correlated with increased preferred body temperatures in the fish. Finally, Magdalena Chadzinska (Jagiellonian University, Poland) used the same system to explain why stress responses can dampen adaptive cell types while increasing circulating neutrophils. After experimentally inducing a stress response, results showed that increased cortisol results in the upregulation of chemokines and cell receptors that leads to the release of stored neutrophils from the head kidney.

12. Evolution and origins of the immune system

The session opened with Martin Flajnik's (Universiy of Maryland, USA) retrospective and tribute to the work of Dr. Gary W. Litman. Dr. Litman made seminal contributions to molecular comparative immunology. First demonstrating adaptive immunity in lamprey, and giving the first reports of the agnathan adaptive receptors latter definitively identified and named variable lymphocyte receptors (VLR) by Zeev Pancer and Max Cooper. Subsequently, in the years before PCR, Litman using cross hybridization to identify an ectothermic IgMH using mouse IgVH. Although many researchers attempted this technique, only Litman was able to successfully identify an immunoglobulin in sharks by this method. This still left many questions of ectothermic adaptive immunity, and Gary Litman contributed many more answers, including: describing the cluster organization of shark Ig genes; demonstrating somatic hypermutation in shark IgH; and isolation of the first shark TCRbeta using a novel short-primer PCR technique (in the face of speculation that sharks may not have T cells or MHC). Finally, Gary Litman's generosity in providing reagents and teaching molecular techniques was hailed as an essential contribution to the growth of comparative immunology.

Anthony De Tomaso (University of California Santa Barbara, USA) presented recent work unraveling the histocompatibility network present in the tunicate *B. schlosseri* as discussed in the context of self-vs. non-self-recognition evolution. Analogous to that used by mammalian natural killer cells to recognize 'rogue' endogenous cells, *B. schlosseri* uses a highly polymorphic gene called *fuhc* which is integral to signaling pathways governing the phenomena of accepting or rejecting colony fusion events when two genetically distinct colonies have physical contact. Expanding upon previous research showing some predictability of fusion events given some measured genetic similarity between *fuhc* alleles of two different colonies, De Tomaso's team have shown that the 'fusability' of colonies can be mutable *in situ* under certain conditions during development.

Jonathan Rast (Emory University, USA) followed with a consolidated overview of immune evolution in the context of the purple urchin (*Strongylocentrotus purpuratus*) and its role in parsing shared deuterostome immune factors from vertebrate-specific immunity components. Rast discussed transcription factors and the specification of the various immune cells in purple urchin larvae, the utility of using purple urchin as a model for understanding the evolution of immunity, and differential expression of immunity-related genes during infection. Ultimately, Rast expressed the importance of investigating novel, even lineage-specific, immune factors present among invertebrate deuterostomes and their fundamental role for understanding the ancestry and evolution of immunity.

Next, Larry Dishaw (University of South Florida, USA) discussed variable-region chitin-binding proteins (VCBPs) and their emerging functions in the chitin-rich gut of *Ciona robusta* (Tunicata). Given the structure of the proteins, Dishaw and his colleagues suspect VCBPs play a role in the immobilization of gut bacteria as well as shaping and maintaining a specific community of gut microflora.

Sebastian Fugmann (Chang Gung University, Taiwan) presented his talk on novel immune genes discovered in the transcriptome of *Strongylocentrotus purpuratus* coelomocytes. Focusing on homology with the somatic diversifying genes RAG1/2 and AID, Fugmann discussed findings on sequence and molecular evolution of the AIDs/APOBEC family of DNA deaminases in *S. purpuratus,* as well as some additional evidence from a brachiopod – placing their evolutionary origin far before the emergence of Ig-based adaptive immunity. Transcriptomic differential expression analyses were also discussed in the inference of function for AID/APOBEC proteins in purple urchin.

Transitioning from presentations focusing on invertebrate immunity, Jeannine Ott (Texas A&M University, USA) presented her talk on somatic hypermutation in shark T cell receptor. Drawing on comparisons to the well-described multiple V-J rearrangments of T-cell receptor alpha genes in mammal's thymic cortex, this talk investigated findings on homologous somatic hypermutation of the T-cell receptor alpha genes in the nurse shark thymus. Employing *in situ* hybridization in addition to RT-qPCR, this presentation explored the spatiotemporal distribution of AID in various thymic regions. When consolidated with bioinformatic analysis, the findings presented in this talk revealed a novel and emerging role of somatic hypermutation for diversifying thymus-derived T-cell receptor alpha transcripts in shark αβ T-cells.

Yuka Ohta (University of Maryland, USA) next presented on the conservation of natural killer receptor NKp30 family across vertebrates, a finding contrary to the hypothesis that natural killer receptors rapidly modify to combat the rapidly changing viral environment. Primarily focusing on the nurse shark model, her findings showed the genomic localization of NKp30 within the shark MHC, as well as expression of NKp30 not only on natural killer cells, but also in shark T cells – supporting shared ancestry of natural killer cells and T cells in early vertebrates.

In the final talk of the session, Jeffrey Yoder (North Carolina State

University, USA) presented data on the function and evolution of novel immune-type receptors (NITRs) in ray-finned fish. NITRs, though not directly orthologous to natural killer cell receptors (NKRs) in mammals, are predicted to play similar roles to NKRs in lymphocyte lineages for recognizing infected/transformed endogenous cells. Yoder and his colleagues identified a suite of NITRs in teleost fish, as well as the earlier-diverging actinopterygians, bowfins and gars. Furthermore, using mammalian cell transformation with the recovered NITRs from teleosts, Yoder provided experimental evidence supporting similar functions of NITRs and NKRs. Finally, single cell transcriptomics show that NITRs are a powerful molecular marker for natural killer cells in the model species, *Danio rerio*.

13. Workshops

Ben Hanelt (University of New Mexico, USA) opened the workshop entitled "*Communicating science to the public*" with the question, "Why?" Why is it so important to communicate science to the public, and why is it so tough to do it well? As a group, we considered those who epitomized the role of a good science communicator – David Attenborough, Neil deGrasse Tyson, E.O. Wilson, Bill Nye – and the traits they exemplified that made them so effective in this role. A good communicator is enthusiastic, passionate, and humorous, tells a story to explain the science, relates the information to the audience, and uses simple language. These traits are difficult to embody for most scientists because of what Chip Heath calls "the curse of knowledge: when we are given knowledge it is impossible to imagine what it is like to lack that knowledge." However, it is only through this type of communication that the lay public actually hears our science.

Hanelt presented four specific topics for improving science communication: structure (path), speech, delivery, and visual aids. He provided several video examples from his own work on host-parasite interactions and from various TED talks, including "How vultures can help solve crimes" by Lauren Pharr. The workshop continued with a list of suggestions for enhancing audience understanding of scientific research. Most importantly, know your audience and speak to them. Your path should be much less direct when addressing a general audience than a group of scholars. Stimulate interest immediately with a strong entry. Limit jargon and unnecessary detail. Use analogy to describe complicated ideas and verbal words to paint pictures. Importantly, have an effective delivery – be enthusiastic and do not hide behind the lectern. Explain how your work connects to the bigger picture. One suggestion for improving delivery is to record yourself speaking and then watch the recording, noting what works and what is distracting. Visual aids should be helpful and clear. Limit content on slides – too many bullets or cluttered slides can serve as roadblocks to understanding. Finally, when possible build your talk using the assertion - evidence approach: deliver messages, not topics; use visuals to back up your message; and explain visuals verbally *on the spot* rather than writing explanations on the slide.

Led by Christopher Bayne (Oregon State University, USA), the "*Let us mentor one another*" workshop took form as a guided seminar on past, present, and emerging paradigms of mentorship in academia. Over the course of the workshop, Bayne maintained participation from workshop attendees by comparing experiences across various academic institutions, asking questions, "Does your institution require formal mentor-mentee meetings? Are mentees encouraged to provide their own agenda for said meetings? Do the mentorship styles the mentor/mentee align with one another?" And finally, "does your institution openly provide remedial avenues for mentor/mentee relationships which may have decayed into relationships disadvantageous to either/both parties?" Finally, subjects like sexual misconduct and gender (in)equality were addressed in the light of contemporary sociopolitical movements (e.g., *#MeToo*). The workshop was well-received and opened dialogues bringing voice to common issues in mentor/mentee relationships (e.g., difficult conversations, authorship disputes, departmental politics, intellectual property, academic bullying). These dialogues were a step forward for a paradigm that is fundamental to successful science training.

Many workshops with similar topics focus on the hardships that women face in STEM, and one leaves feeling more discouraged than inspired. However, the workshop "*Women in STEM: challenges and opportunities*", facilitated by Irene Salinas and Janeth Peña (both at University of New Mexico, USA), went beyond the usual scope and presented possible solutions for the issues faced by women in STEM fields. The goal of the workshop was not to collectively speak about hardships faced by women in STEM, but rather to understand that while there currently are barriers that will be encountered in a woman's career, through perseverance and the education of both sexes, these obstacles can be overcome and eventually eradicated.

One of the main topics discussed was the need to preach confidence in your own work to both girls and boys in all age groups. A study was presented indicating that as children age, boys tend to approach challenges with more confidence than girls, which is a learned behavior due to the encouragement of boys at a young age. Confidence is equally as important as competence, and it was found that while men would apply for a position or promotion when they believed they could meet 60% of the requirements, women would apply only when they believed they met 100% of the requirements. Salinas stressed that women need to believe in the work that they are doing, become more aggressive when defending their beliefs, talk about their work with pride, and to be confident in asking for help if it is needed. Both the facilitators and attendees highlighted the need for change and expressed that this change has to come from both men and women in order to progress towards equality and the end of discrimination. It was agreed by all in attendance that change needs to come from the top with respect to administration and tenure-track positions. Personal anecdotes from Drs. Salinas and Peña about how it is possible to balance a science career and a family afforded encouragement and relief to the young female scientists in attendance, as this is a common concern. Multiple breakout sessions included small-group discussion about case studies that ranged from a woman discovering her colleagues were only bringing her on to a project because she was a young woman and it would be advantageous for a grant, to blatant and outright sexism experienced when another woman admitted she was pregnant to her PI during her PhD. Each group provided possible solutions to each situation and spoke about what they personally would have done if found in such a predicament.

A crucial portion of the workshop was spent discussing tried and true solutions that allow women to work towards ending inequality in STEM-related fields. Confidence, a reoccurring theme, was most often referenced. It was suggested that women should not be afraid to claim credit for their own ideas, invest in peer networks for support, build up protégés (a sign of leadership skills), and hone their brand while talking about their own work with pride. In the face of failure, it was discussed that persistence is key, especially when resubmitting for grants and publications. Other solutions presented were to be creative with failure, or change your focus if repetition is not yielding results. Finally, it was advised to utilize national and international collaborations to increase opportunities for success if they are not presented locally.

The 14th Congress of the ISDCI came to a close after five days of exciting science and reports of new discoveries. Delegates returned home with fresh ideas, reports of the latest advancements in their field, understanding of new discoveries and memories of great fun with old friends and new collaborators. The future of comparative immunology looks very bright indeed.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dci.2019.02.016.

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