PROCEEDINGS

24th JAPANESE ASSOCIATION FOR DEVELOPMENTAL & COMPARATIVE IMMUNOLOGY

Fukuoka　Japan

July 9 to 14, 2012

日本比較免疫学会

第 24 回 学術集会講演要旨

会 期：２０１２年７月９日（月）～１３日（金）
会 場： ヒルトン福岡シーホーク･ホテル

学 術 集 会 会 長：中尾　実樹（九州大学）
学術集会 事務局長：杣本　智軌（九州大学）

日本比較免疫学会

―２０１２―

会場：ヒルトン福岡シーホーク

810-8650 福岡市中央区地行浜 2-2-3

TEL 0192-844-8111/FAX 092-844-7887

福岡空港から

- 地下鉄で

  福岡空港駅から乗車　⇒　西新駅または唐人町駅下車　⇒　西新・唐人町駅よりホテルへは徒歩約19分・タクシー約6分・バス約6分

  唐人町からの地図　／　唐人町からのバス時刻表（西鉄ホームページへ）

  西新からの地図　／　西新からのバス時刻表（西鉄ホームページへ）

- バスで

  バス停「福岡空港国内線」から【福岡タワー南口TNC(行き先番号39番)】乗車（約40分）　⇒　シーホーク前バス停下車　徒歩約1分

  福岡空港からの時刻表（西鉄ホームページへ）

- タクシーで

  福岡都市高速道路を経由した場合、乗車時間　約20分

- 空港からのおすすめのルート

  空港～博多駅（地下鉄5分）博多駅バスセンター～シーホークホテル前（西鉄バス約20分）です。

博多駅から

- 地下鉄で

  博多駅より乗車　⇒　唐人町駅もしくは西新駅にて下車　⇒　西新・唐人町駅よりホテルへは徒歩約19分・タクシー約6分・バス約6分

  唐人町からの地図　／　唐人町からのバス時刻表（西鉄ホームページへ）

  西新からの地図　／　西新からのバス時刻表（西鉄ホームページへ）

- バスで

  バス停「博多バスターミナル」6番乗り場から【福岡タワー南口TNC(行き先番号306番)】【「藤崎」（行き先番号306番）】に乗車　⇒　シーホーク前バス停下車　徒歩約1分

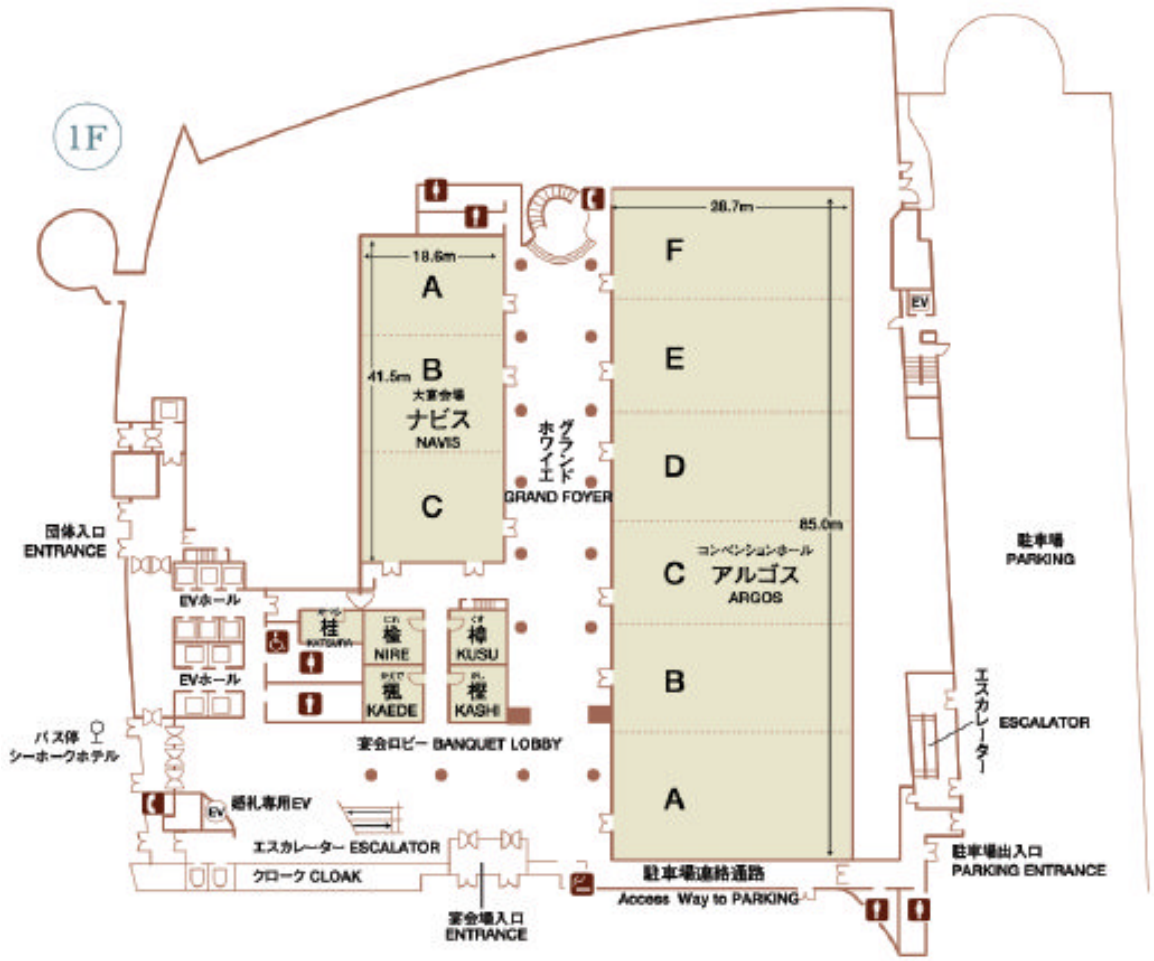
  博多駅からの時刻表（西鉄ホームページへ）

- タクシーで

  福岡都市高速道路を経由した場合、 乗車時間　約15分

館内見取図

1階平面図



NAME OF ROOM		コンベンション・大宴・ホワイエ ARGOS+NAVIS+FOYER	コンベンションホール アルゴス(ARGOS)						大宴会場 ナビス(NAVIS)		
		全室	全室	5/6	4/6	3/6	2/6	1/6	全室	2/3	1/3
面積 DIMENSION	㎡	4,114	2,440	2,043	1,650	1,214	795	408	777	517	253
	坪数	1,244	739	619	500	368	240	123	233	156	76

NAME OF ROOM		小宴会場				VIP控室
		楡 NIRE	楓 KAEDE	楠 KUSU	樫 KASHI	桂 KATSURA
面積 DIMENSION	㎡	53	51	56	55	46
	坪数	16	15	17	17	14

The Symposium of
the 3 Japanese Societies;

***Japanese Association for Developmental & Comparative Immunology (JADCI)**

***Japan Society for Comparative Endocrinology (JSCE)**

***Japanese Society for Comparative Physiology & Biochemistry (JSCPB)**

Recognition Mechanisms

-Current Topics in Comparative Biology-

Organized by Dr. Haruhisa Wago (Saitama Medical University)

July 9th, 2012 (PM 3:30-18:00)
at Hilton Fukuoka Sea Hawk Hotel, Fukuoka City

JADCI

Recognition of Pathogenic or Symbiotic Bacteria and Immune Responses in Insects

Lee Bok Luel

The Global Research Lab (GRL) for Insect Symbiosis and National Research Laboratory (NRL) of Host Defense Proteins, College of Pharmacy, Pusan National University, Jangjeon Dong, Kumjeong Ku, Busan, 609-735, Korea, E-mail: brlee@pusan.ac.kr

The insect's Toll receptor signaling pathway is responsible for the defense against Gram-positive bacteria and fungi, while the immune deficiency (Imd) pathway is activated primarily in response to Gram-negative bacteria. Both of these pathways lead to the expression of antimicrobial peptides (AMPs) via the activation of NF-κB-like transcription factors. Even though elegant *Drosophila* genetic studies characterized the innate immune responses in detail, there is some limitation to reveal the biochemical mechanisms underlying invertebrate's innate immune responses. To investigate the biochemical basis of how insects recognize invading pathogenic microbes and how these recognition signals are transferred to the down-stream factors and activate host innate immune responses, it is necessary to use large insects, from which larger amounts of hemolymph (insect blood) can be extracted and can be used to perform biochemical studies. We have recently determined molecular activation mechanisms of insect's Toll signaling pathway after purification of several pattern recognition proteins, serine proteases, serpins and AMPs. In this talk, we will discuss the biochemical characterization of these molecules, which are involved in the recognition of pathogenic microbes, in the amplification of recognition signal transduction and in the regulation of proteolytic cascade leading to the production of AMPs in insects.

Also, I will discuss the molecular mechanisms of how insect hosts distinguish pathogenic bacteria and symbiotic bacteria in order to defense their bodies in insects.

JADCI

Immunological Recognition in Starfish Larvae and Adults: The Alteration of Immune Cells Via Metamorphosis

R. Furukawa[1, 2]

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[2] School of Fundamental Science and Technology, Graduate School of Science and Technology, Keio University, Japan

The starfish is a phylogenetically primitive animal, and hence provides us insight into the ancestral immune system during evolution of the deuterostomes. I have been characterizing the immune cells of the starfish larva, about which there has been little progress since the discovery of phagocytosis by Metchnikoff. In these immune cells, there is the apparent replacement of the larval mesenchyme cells with the adult coelomocytes via metamorphosis. In this symposium, I will focus on the self/nonself recognition by both types of immune cell.

I have demonstrated that self recognition is strikingly altered in the two types of immune cell via metamorphosis. The larval mesenchyme cells selectively phagocytose xenogeneic sperm, when live allogeneic and xenogeneic sperm are simultaneously injected into the body. This result indicates that the larval mesenchyme cells recognize sperm of the same species as a self. In other words, they lack the allorecognition capability. Interestingly, the larval mesenchyme cells phagocytose live allogeneic sperm treated with a mixture of various glycosidases. In the adult, the aggregate formation of coelomocytes occurs in response to allogeneic transplantation of coelomocytes, but not in response to autotransplantation. This observation strongly suggests that the allorecognition capability is acquired after metamorphosis in the starfish immune system.

Recently, I have identified a novel opsonin against bacteria, ApSRCR1, as one of the nonself recognition molecules. ApSRCR1 is expressed in both the larval mesenchyme cells and adult coelomocytes. Significantly, ApSRCR1 differs in its activity as an opsonin between the two types of cells. Although functional inhibition of ApSRCR1 leads to incomplete phagocytosis, this inhibitory effect is much higher in the coelomocytes than in the mesenchyme cells. In addition, biochemical analysis shows that ApSRCR1 exhibits not only higher expression, but also a higher extent of modification by N-glycosylation in the coelomocytes than in the mesenchyme cells. Thus, the importance of a certain type of immune molecule is altered in the immune system with alteration of the immune cells via metamorphosis. In addition to these findings, I will present a global analysis comparing immune-related genes expression in the larval mesenchyme cells and the adult coelomocytes.

JSCE

Comprehensive Analysis of Neuropeptides of Marine Invertebrates

S. Kato[1], K. Ohno[2], A. Fujiwara[3], M. Awaji[4], T. Matsumoto[4], K. Yamano[4], M. Yoshikuni[1]

[1] Department of Bioscience and Biotechnology, Kyushu University, Japan
[2] National Institute for Basic Biology, USA
[3] National Research Institute of Fisheries Science, JAPAN
[4] National Research Institute of Aquaculture, JAPAN

It has been emerging that neural systems of marine invertebrates produce a large number of secretable peptides which may play important roles in the control of various biological phenomenon. This progress highly depends on the improvement of analytical instruments such as a DNA sequencer and a mass spectrometer. Currently, we can identify a lot of peptides from neural tissues of only a few individuals by using these updated instruments.

Comprehensive analysis of mRNAs provides a list of possible secretory peptides with a confirmation of a signal sequence and convertase-cleaving sites. But, it will not give a list of actually-secreted peptides. A mass spectrometer can directly detect small quantities of peptides expressed in tissue samples. However, it will require repetitive laborious analyses for obtaining accurate identification of peptides.

The combined use of mRNA analysis and mass spectrometry compensates imperfections of each method to help rapid and accurate detections of secretory peptides. We comprehensively analyzed ESTs and peptides of radial nerves of red sea urchin (*Pseudocentrotus depressus*) and visceral ganglions of oyster (*Crassostrea gigas*). We have identified many peptides over a hundred including large numbers of unknown sequences in each animal by the combined use of EST and mass spectrometry. Some of those peptides were interestingly classified into several groups. These groups are characterized with unique sequences of amino acids at the carboxyl-terminal ends, respectively.

Currently, the use of next generation DNA sequencer rapidly accelerates increase in number of registered sequence data at the SRA archive site of NCBI. The combined use of EST and MS will be more powerful to identify new peptides and establish an information infrastructure for neuroendocrinology.

JSCE

Ligand recognition and signal transduction of GPCRs and Toll-like receptors of the protochordate, *Ciona intestinalis*: what is evolutionarily common or unique?

H. Satake

Bioorganic Research Institute, Suntory Foundation for Life Sciences, Japan

Ascidians, invertebrate deuterostomes, belong to the subphylum Tunicata or Urochodata in the phylum Chordata. Their critical phylogenetic position as protochordates suggests that they conserve evolutionary origins of various biological systems of vertebrates: nervous, neuroendocrine, endocrine and innate immune systems. Over the past few years, the cosmopolitan species, *Ciona intestinalis*, has been shown to possess orthologs or prototypes of vertebrate neuropeptides, peptide hormones, and their receptors including gonadotropin-releasing hormones (GnRHs) and their receptors. To date, seven GnRH family peptides (t-GnRH3 to -8 plus Ci-GnRH-X) and four their receptors (Ci-GnRHR1 to -4) have been identified in *C. intestinalis*. t-GnRHs, like vertebrate counterparts, activate Ci-GnRHRs in a ligand-selective manners, whereas Ci-GnRH-X moderately inhibits the activities of t-GnRHs at Ci-GnRHR1 and -3. A striking feature of t-GnRH signaling is that the *Ciona*-specific GnRHR orphan paralog, Ci-GnRHR4, heterodimerizes with Ci-GnRHR1 and -2, leading to the differential regulation of signal transduction via the two receptors without ligand binding affinity altered. These findings reveal that *C. intestinalis*, which lacks a pituitary (a major target of hypothalamic GnRH in vertebrates), conserves GnRH signaling essentially common to vertebrates and established the species-specific signaling regulatory mechanisms.

C. intestinalis has also been found to possess Toll-like receptors (TLRs) that play crucial roles in innate immunity of vertebrates. Intriguingly, only two TLR genes are present in the *C. intestinalis* genome, while other deuterostome invertebrates, sea urchin and amphioxus harbor 222 and 72 TLR candidates. Of particular interest is that the two *C. intestinalis* TLRs, i.e., Ci-TLR1 and -2, activate NF-κB in response to multiple TLR ligands which are recognized by different mammalian TLRs: zymosan for human TLR2 (hTLR2), heat-killed *Legionella pneumophila* for hTLR2, and poly(I:C) for hTLR3. These data verify both common and unique functionality of Ci-TLRs, compared with mammalian orthologs. In addition, such recognition by Ci-TLRs cannot be predicted by sequence homology-based search, suggesting the molecular diversity in ligand recognition by non-vertebrate TLRs. Collectively, species-specific multiplication and loss of genes for endogenous ligands and receptors, including peptides, GPCRs, and TLRs, generate various and unique molecular recognition, which is likely to be implicated in the evolution and diversity of organisms.

JSCPB

OPN5, a Photosensory Protein for Mammalian Ultraviolet Photoreception

D. Kojima

Department of Biophysics and Biochemistry, Graduate School of Science,
The University of Tokyo; PRESTO, Japan Science and Technology Agency, Japan

The ultraviolet (UV) component of sunlight is utilized in a variety of animal species for their environmental cues, e.g., for flower discrimination and orientation/navigation in insects and for mate choice and parent-offspring communication in birds: Consistently, these animals have UV-sensitive photoreceptors in their eyes. In mammals, some rodents such as mice have UV-sensitive retinal cone photoreceptor cells, whose function remains yet unknown, while most other mammalian species including human beings have been believed to lack such UV photoreception mechanisms. Here I report that mammalian OPN5 protein (neuropsin) is a UV-sensitive photoreceptor. The *Opn5* gene encoding OPN5 protein has been first identified in the mouse and human genomes as a member of rhodopsin (opsin) family, most of which encode photosensitive proteins. The mouse *Opn5* mRNA expression is detected in various neural tissues, though its detailed cellular expression pattern has not been reported. Furthermore, it has remained unclear whether OPN5 functions as a GPCR-type photopigment or photoisomerase, or has functions other than those. This study was aimed to examine molecular function of mammalian OPN5 and its detailed expression pattern in the tissues.

Mouse OPN5 protein bound a chromophore 11-*cis*-retinal and exhibited an absorption maximum at 380 nm. Upon UV-light illumination, OPN5 was converted to a blue-absorbing photoproduct, which was stable in the dark and reverted to the UV-absorbing state by the subsequent orange light illumination, indicating its bistable nature. Human OPN5 also had the absorption maximum at 380 nm with spectral properties similar to mouse OPN5, revealing that OPN5 is the first and hitherto unknown UV light-sensitive opsin in human beings. OPN5 had the ability to activate hetrotrimeric G-protein Gi in a UV-dependent manner. Immuno-blotting analyses of mouse tissue extracts identified retina, brain and, unexpectedly, outer ear as the major sites of OPN5 expression. In the mouse tissue sections, OPN5 were detected in a subset of non-rod, non-cone retinal neurons as well as in the epidermal and muscle cells of the outer ear. Most of these OPN5 immuno-positive signals were co-localized with the Gi-positive signals. These results demonstrate the first example of UV-sensitive photoreceptor in human beings and strongly suggest that OPN5 transduces UV-sensitive Gi-mediated signaling pathway in the mammalian tissues.

JSCPB

Specificity and Sensitivity of Sex Pheromone Perception in the Silkmoth *Bombyx mori*

T. Sakurai, M. Tabuchi, R. Kanzaki

Research Center for Advanced Science and Technology, The University of Tokyo, Japan

Male moths locate their mates using species-specific sex pheromones emitted by conspecific females. One striking feature of sex pheromone recognition in male moths is the high degree of specificity and sensitivity at all levels from primary sensory processes to behavior. Despite advances in our understanding of molecular, structural, and physiological mechanisms underlying pheromone detection at peripheral level, mechanisms that define behavioral response specificity to conspecific pheromones and the ability to display behavioral responses to minuscule amounts of pheromone molecules are still obscure. In this talk, recent work that addresses these two questions using a genetically tractable moth species the silkmoth, *Bombyx mori*, will be presented.

In the silkmoth a single pheromone component, (*E,Z*)-10,12-hexadecadienol (bombykol), is sufficient to elicit full sexual behavior in males. The sex pheromone receptor BmOR1 expressed in specialized olfactory receptor neurons (ORNs) mediates specific detection of bombykol in the antennae of male silkmoths. To examine causal relationships between sex pheromone receptor specificity and behavioral specificity, we genetically expressed the sex pheromone receptor PxOR1 of the diamondback moth *Plutella xylostella* in BmOR1-expressing ORNs. Expression of PxOR1 conferred the ability to respond to its specific ligand, (*Z*)-11-hexadecenal, at electrophysiological and behavioral levels. These results indicate that activation of BmOR1-expressing ORNs alone is sufficient to trigger full sexual behavior. Thus, behavioral specificity is determined by sex pheromone receptor ligand specificity in the silkmoth.

We also examined how the sensitivity of ORNs is processed in the brain to generate high behavioral responsiveness. To precisely control the activity of BmOR1-expressing ORNs, we genetically introduced a light-gated ion channel, channelrhodopsin-2, in these ORNs. Using paired-pulse photostimulation at various interstimulus intervals, we found that ORN activity that is subthreshold in terms of behavior can be amplified to suprathreshold levels by temporal integration in antennal lobe projection neurons (PNs) if occurring within a specific time window. Temporal integration in PNs was only observed for weak inputs but not for strong inputs. These results show that temporal integration of ORN inputs in PNs contributes to high behavioral sensitivity by lowering the threshold of behavioral responsiveness.

Oral presentation

Session: Anti viral immunity

Functions of CD8-positive and CD4-positive lymphocytes against virus-infection in ginbuna crucain carp

Tomonori Somamoto[1], Teruyuki Nakanishi[2], Miki Nakao[1]

[1] *Department of Bioscience and Biotechnology, Kyushu University, Japan*
[2] *College of Bioresource Sciences, Nihon University, Japan*

Recent studies have shown that cytotoxic and helper T-cell functions are well-conserved in teleost fish. However, information on their functions against virus-infection is still limited. The present study demonstrated the functions of CD4-positive and CD8-positive lymphocytes employing clonal ginbuan crucian carp, anti-ginbuna CD8**α** and CD4 mAb and crucian haematopitetic necrosis virus (CHNV). The cytotoxic assays using syngeneic cell line as a target showed that CD8-positive lymphocytes from CHNV-sensitized fish are significantly capable of killing infected-syngeneic target cells. The inhibition of the activity by concanamycin A suggests that virus-specific CTL kill virus-infected cells utilizing perforin/granzyme pathway. However, the activity of CD8-positive cells is comparable or lower than that of monocytes-enriched effector cells or CD8-negative lymphocytes. This finding suggests that CTLs may be not dominant effector cells that are induced by CHNV-infection. To confirm whether helper T-cells (Th cells) of teleost fish actually facilitate secondary humoral and cellular immune responses, the adoptive transfer study was performed using clonal donors and recipients. Naïve recipients receiving CHNV-sensitized donor cells started to produce specific antibody at 5 days post infection (dpi), while antibody production in the recipients receiving non-sensitized cells or CHNV-sensitized Th cell-depleted cells became detectable at 12 dpi. This finding suggests that the adoptive transfer of Th cells provide the recipient faster antibody response similar to secondary ones. On the other hand, although adoptive transfer of sensitized donor cells into naïve fish induced a significant enhancement of cell-mediated immunity, no significant difference of the cell-mediated immune response was observed in the recipients receiving the sensitized donor cells with or without Th cells. These results indicate that Th cells of teleost fish can help B-cells, but it remains unclear whether they can help CTLs. Thus, further study would be needed to understand the induction system of humoral and cell-mediated immunity in teleost fish.

Session: Complement and Complement-like factors

Cytolytic factor in the mosquito

Toshinori Sasaki, Mutsuo Kobayashi

Department of Medical Entomology, National Institute of Infectious Diseases,

Shinjuku-ku, Tokyo, Japan, 162-8640

Although cytolysis of invading organisms is an innate form of immunity used by invertebrates, so far the underlying mechanism remains less explored. The pupal hemolymph of the mosquito *Armigeres subalbatus* induces an activity that causes hemolysis of human red blood cells (HRBC). This hemolytic activity was inhibited by sialic acid (N-acetylneuraminic acid) and serine protease inhibitors. We purified the sialic acid-specific lectin(s) from the pupal hemolymph using formaldehyde-fixed HRBC and determined the sequence of the amino-terminal 19 amino acid residues. A polyclonal antibody produced against this N-terminal peptide clearly inhibited the hemolytic activity of the hemolymph *in vitro*, thus suggesting that the hemolysis of HRBC is caused by the lectin present in the mosquito hemolymph. We suggest that mosquitoes possess a cytolysis system.

Isotypic Diversity in the Ontogenetic Expression of the Complement Component in the Common Carp (*Cyprinus carpio*)

Vo Kha Tam[1], Chie Okura[1], Masakazu Kondo[2], Tomonori Somamoto[1], Miki Nakao[1]

[1] *Department of Bioscience and Biotechnology, Kyushu University, Japan.*
[2] *Department of Applied Aquabiology, National Fisheries University, Shimonoseki 759-6595, Japan.*

Isotypic diversity of complement components is a striking feature of teleost complement system. As a first line of innate defense, the complement system has been considered as an important clue of humoral defense in early development, however functional diversity of the isotypes during teleost ontogeny is poorly understood. The present study aimed at clarifying comprehensive picture of ontogenetic expression of the diversified complement component isotypes in carp. Real-time quantitative PCR detected embryonic expression of C1r/s, MASPs, factor B/C2, C3, C4, C5, C6, C7, C8, C9, and factor I. A remarkable difference in the expression time course was noted between the isotypes in C3, C4, and C5. Especially, teleost-specific isotypes of C3 and C4 (non-histidine-type) started around hatching, in contrast to evolutionarily common isotypes (histidine-type), which showed much earlier expression. Whole-mount in situ hybridization of carp embryos revealed some difference in embryonic expression sites of two major C3 isotypes (C3-H1 and C3-S) in addition to common expression sites such as the yolk syncytial layer. The temporal and spatial differences in expression among the isotypes suggest that the isotypes are functionally differentiated in teleost early development.

Identification of Pattern-Recognition Molecule in Hagfish Complement System

Tomokazu Yamaguchi[1], Kazufumi Takamune[1], Masakazu Kondo[2],
Yukinori Takahashi[2],Yoko Kato-Unoki[3], Miki Nakao[4], Tamotsu Fujii[5]

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[2]*Department of Applied Aquabiology, National Fisheries University, japan*
[3]*Faculty of Agriculture, Kyushu University, japan*
[4]*Department of Bioscience and Biotechnology, Kyushu University, japan*
[5]*Department of Health Sciences, Hiroshima Prefectural University, japan*

All extant jawed vertebrates share a common adaptive immune system in which immunoglobulin domain-based molecules act as antigen receptors. On the other hand, jawless vertebrates, lamprey and hagfish, use variable lymphocyte receptors composed of leucine-rich repeat cassettes for antigen recognition. Since invertebrates and primitive chordates do not have adaptive immune system, immune system seems to change dramatically in course of evolution from primitive chordate to gnathostome. From a phylogenic perspective of defense mechanisms, previously we found complement C3 and mannose-binding lectin-associated serine protease 1 (MASP-1) in hagfish and suggested the involvement of lectin pathway in hagfish innate immune system. In this study, we focused on the pattern recognition molecules in the hagfish, *Eptatretus burgeri*, and tried to purify it from serum by affinity chromatography. When the serum was treated with GlcNAc-agarose, followed by successive elution of the binding molecules with GlcNAc and EDTA, mainly four proteins (31 kDa, 27 kDa, 26 kDa, and 19 kDa) and 26 kDa protein were detected in the GlcNAc-eluate and EDTA-eluate, respectively. Collagenase treatment showed the presence of collagen-like domain only in the 26 kDa protein in the EDTA-eluate. Since common pattern recognition molecules such as mannose-binding lectin possess collagen-like domain, we examined the entity of the 26 kDa protein by sequencing its N-terminal amino acids and cDNA obtained by 3' and 5' RACE methods, and identified it as a member of C1q family. Herein, it will be referred to as hagfish C1q (hagC1q). Western blot analyses using anti-hagC1q, MASP-1, and complement C3 antibodies showed that hagC1q associated with MASP-1 and complement C3 in the serum and had binding ability to *Escherichia coli* as a divalent cation-dependent manner. These results suggest that hagC1q plays an important role in hagfish innate immune system.

Microbe-specific C3b deposition in the horseshoe crab complement system in a C2/factor B-dependent or -independent manner

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[2] Department of Biology, Faculty of Sciences, Kyushu University, Japan
[3] Department of Immunology, Fukushima Medical University School of Medicine, Japan

Complement C3 plays an essential role in the opsonization of pathogens in the mammalian complement system, whereas the molecular mechanism underlying C3 activation in invertebrates remains unknown. To understand the molecular mechanism of C3b deposition on microbes, we characterized two types of C2/factor B homologs (designated TtC2/Bf-1 and TtC2/Bf-2) identified from the horseshoe crab *Tachypleus tridentatus*. Although the domain architectures of TtC2/Bf-1 and TtC2/Bf-2 were identical to those of mammalian homologs, they contained five-repeated and seven-repeated complement control protein domains at their N-terminal regions, respectively. TtC2/Bf-1 and TtC2/Bf-2 were synthesized and glycosylated in hemocytes and secreted to hemolymph plasma, which existed in a complex with C3 (TtC3), and their activation by microbes was absolutely Mg^{2+}-dependent. Flow cytometric analysis revealed that TtC3b deposition was Mg^{2+}-dependent on Gram-positive bacteria or fungi, but not on Gram-negative bacteria. Moreover, this analysis demonstrated that Ca^{2+}-dependent lectins (C-reactive protein-1 and tachylectin-5A) were required for TtC3b deposition on Gram-positive bacteria, and that a Ca^{2+}-independent lectin (*Tachypleus* plasma lectin-1) was definitely indispensable for TtC3b deposition on fungi. In contrast, a horseshoe crab lipopolysaccharide-sensitive protease factor C was necessary and sufficient to deposit TtC3b on Gram-negative bacteria. We conclude that plasma lectins and factor C play key roles in microbe-specific TtC3b deposition in a C2/factor B-dependent or -independent manner.

Session: Cytokine and Chemokine

A chemokine expressed in the secondary lymphoid organs in of fugu (*Takifugu rubripes*)

H. Tauchi[1], H. Suetake[1, 2], T. Odaka[2], M. Kaneda[2], T. Maeda[2], K. Kikuchi[1], Y. Suzuki[1], T. Miyadai[2]

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secondary lymphoid organs differ substantially from those of mammals. They are composed of the spleen and kidney and lack lymph nodes and germinal centers. Understanding adaptive immune responses in fish requires detailed characterization of the microarchitecture of their secondary lymphoid organs. Lymphoid chemokines such as CCL19 and CCL21 regulate trafficking of lymphocytes and antigen-presenting cells to secondary lymphoid organs, facilitating adaptive immune responses. In the present study, we surveyed the fugu genomic database and found 3 CCL19 or CCL21-like genes. Two of the 3 genes encode a CC chemokine resembling CCL19, with 4 cysteines, and they are evidently duplicated genes, as there is only a single nucleotide difference within their coding regions. The third gene encodes a CC chemokine including 6 cysteines such as mammalian CCL21. This chemokine was expressed in the spleen and kidney, whereas the other 2 were not expressed in these organs. This indicates that this chemokine is the secondary lymphoid chemokine in fish. *In situ* hybridization studies showed that this lymphoid chemokine was expressed in melanomacrophage centers (MMCs). These results suggest that fish MMCs play pivotal roles in adaptive immune responses.

Identification, characterization and expression analysis of IL-17 receptors, Act1 and TRAF6 genes in Japanese pufferfish (*Takifugu rubripes*)

H. Korenaga[1], T. Kono[2] , M. Sakai[1]

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Interleukin-17 (IL-17) is the novel pro-inflammatory cytokine family with six members (A-F) that defines a new class of CD4effector T cells, termed as Th17. Teleost IL-17 was reported to consist of seven ligands including teleost specific member, IL-17N in some species. However, IL-17 receptors and their signaling are few reported in teleosts. In mammals, IL-17 receptors (5 members, RA-RE) have a cytoplasmic tail with motifs similar to the TIR superfamily, now termed SEF/IL−17R (SEFIR) domain. Act1 interacts with the IL-17 receptors through the C-terminal SEFIR domain. In addition to interacting with the SEFIR domains of IL-17 receptors, Act1 also functions as an adaptor protein by binding to additional signaling molecules, most notably recruitment of TNF receptor associated factor 6 (TRAF6) by two putative TRAF-binding sites. This signaling activates the NF-kB transcription factor pathway. In this report, we identified seven IL-17 receptors (RA, RC-1, -2, RD-1, -2, -3 and RE), Act1 and TRAF6 from Japanese pufferfish, *Takifugu rubripes* using comparative genomics based on chromosome synteny and characterized their biological activities. *Fugu* IL−17RC1 and RE genes harbored on scaffold_180 and other *Fugu* IL-17 receptors were found to be located on independent contigs. *Fugu* IL-17 receptors showed single transmembrane domain-containing receptors, ranging in size from 470 to 755 amino acid. Moreover, these receptors contained certain conserved the SEFIR domain. *Fugu* Act1 contained TRAF binding sites at the N-terminus, and Act1 had a SEFIR domain located at the C-terminus. *Fugu* TRAF6 ORF is predicted to encode 561 amino acid. Multiple alignment with other known TRAF6 genes revealed conservation among vertebrates. The expression pattern of *Fugu* IL-17 RA and RC genes were observed in almost all organs, while the other *Fugu* IL-17 receptor genes expressed in few organs. In particular, IL-17 receptor genes showed highly expression in the spleen. Moreover, expression of IL-17 receptor genes showed different pattern in the mitogen-stimulated head kidney cells. In addition, expression analysis of *Fugu* Act1 and TRAF6 genes may play important roles in inflammatory responses in fish.

Session:Ig and other antigen receptors

VLRC defines a population of lamprey lymphocytes distinct from that expressing VLRA or VLRB

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Instead of T-cell and B-cell receptors (TCR and BCR), jawless vertebrates such as lamprey and hagfish use variable lymphocyte receptors (VLRs) as antigen receptors. VLRs generate diversity comparable to that of TCR or BCR by assembling highly diverse leucine-rich repeat (LRR) modules presumably via cytidine deaminases. Like TCR and BCR, they appear to exhibit allelic exclusion with each lymphocyte expressing a unique VLR molecule. We have previously shown that VLR monomers adopt a horseshoe-shaped structure characteristic of LRR family proteins and that hypervariable residues are located predominantly on the concave surface, suggesting that VLR binds antigen on its concave surface. This suggestion was confirmed by the crystallographic analysis of lamprey VLR molecules in complex with the H-trisaccharide from human blood type O erythrocytes. Both hagfish and lampreys have two types of VLRs, named VLRA and VLRB. VLRB occurs as pentamers or tetramers of dimers and constitutes major agglutinating antibodies produced in response to antigen stimulation. On the other hand, VLRA is a membrane-bound receptor not secreted into the serum. Recent evidence indicates that lamprey VLRA+ and VLRB+ cells display gene expression profiles resembling those of T- and B-cells of jawed vertebrates, respectively, suggesting that lampreys also have a dual antigen receptor system analogous to that of jawed vertebrates. Here, we describe the identification of a third lymphocyte lineage expressing a distinct antigen receptor, VLRC. Single cell genomic PCR indicates that VLRC is assembled in a population of lymphocytes distinct from VLRA+ or VLRB+ cells. The apparent presence of a transmembrane region and the failure to detect a secreted form indicate that VLRC is a membrane-bound antigen receptor. Thus, like jawed vertebrates that have B-cells and two major populations of T-cells ($\alpha\beta$ and $\gamma\delta$ T cells), lampreys appear to have three lineages of lymphocyte-like cells, of which one is B-cell-like and two are presumably T-cell-like.

Session: Innate Cellular immunity

Phagocytosis and bactericidal abilities of teleost thrombocytes

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Thrombocytes have been recognized as hemostatic cells in nonmammalian vertebrates. Unlike mammalian platelets, thrombocytes are nucleated cells with a lymphocyte-like morphological feature, and possible involvement of thrombocytes in innate immune function has been considered in addition to the hemostatic function. In the present study, we report phagocytic abilities of some teleost thrombocytes. Using a monoclonal antibody specific for thrombocytes of common carp (*Cyprinus carpio*), thrombocytes were isolated from peripheral blood and examined for expression of various immune-related genes, resulting in detection of significant level of lysozyme, iNOS and MHC class II using RT-PCR. Upon flow cytometry-based phagocytosis assay, a number of thrombocytes ingested fluorescent latex particles (0.5 μm, 1 μm, 2 μm and 3 μm), bacteria (*Escherichia coli*) and zymosan particles. Phagocytosis by the thrombocytes was also confirmed by fluorescent microscopy and transmission electron microscopy, which revealed internalization of these particles into thrombocytes. We also observed that thrombocytes of the olive flounder (*Paralichthys olivaceus*) had similar phagocytic behavior. These data indicate that thrombocytes of those species are potent phagocytes, suggesting that those phagocytic characteristics of thrombocytes are widely conserved in teleosts. We also assessed a phagolysosome formation ability of teleost thrombocytes against the ingested pathogens, for detecting intracellular bactericidal activities of those thrombocytes. By using an ImageStream multi-spectral flow cytometer, we assessed phagolysosome fusion of goldfish (*Carassius auratus*) thrombocytes. On this analysis we detected that lysosomes of these thrombocytes visualized with fluorescent dextran were colocalized the ingested small beads and were formed a ring around a large beads like other typical phagocytes. Overall, the results indicate that teleost thrombocytes have dual functions as not only hemostatic cells but also as phagocytic immune cells against microbial infections.

Session: Invertebrate immune responce

Transglutaminase-catalyzed Relish Crosslinking Suppresses Innate Immune Signaling in the *Drosophila* Gut

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Mammalian transglutaminases (TGs) play important roles in numerous physiological phenomena such as blood coagulation and skin formation via protein-protein crosslinking. We show that *Drosophila* cytoplasmic TG suppresses innate immune signaling in the gut. *TG*-RNA-mediated interference (*TG*-RNAi) caused a short life span under non-sterile conventionally reared conditions, but not under germ-free conditions. Under conventionally reared conditions, *TG*-RNAi enhanced the expression of immune deficiency (IMD) pathway-controlled antimicrobial peptide genes. Ingestion of gut lysates prepared from conventionally reared *TG*-RNAi flies into non-*TG*-RNAi flies resulted in the short life span of the recipients. *TG*-RNAi under conventionally reared conditions triggered severe apoptosis in the gut and induced the translocation of Relish, the NF-κB-like transcription factor of the IMD pathway, from the cytoplasm to the nucleus. Ingestion of synthetic amine donors by non-*TG*-RNAi flies, which inhibits the TG-catalyzed protein-protein crosslinking reaction, also induced the nuclear translocation of Relish and enhanced the expression of IMD-controlled antimicrobial peptide genes in the gut. We conclude that TG-catalyzed Relish crosslinking suppresses the IMD-signaling pathway to maintain a buffered threshold required for immune tolerance against commensal microbes.

Session: T-cell, MHC, and Ag-presentation

Long-term Transspecies Dimorphism of the *PSMB8* Gene in the Actinopterygian MHC Region

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The proteasome subunit beta type-8 (*PSMB8*) gene encodes a catalytic subunit of the immunoproteasome, which is involved in the generation of peptides presented by MHC class I molecules. Two highly divergent types of *PSMB8* termed the A and F types, possessing Ala or Val and Phe or Tyr, respectively, at the 31st residue of the mature peptide located in the S1 pocket, have been identified in amphibian *Xenopus* species, teleost species belonging to Cypriniformes (zebrafish), Salmoniformes (trout), Beloniformes (*Oryzias* species), and Tetradontiformes (pufferfish), and elasmobranch sharks. Phylogenetic analysis indicated the presence of two very ancient lineages, the *PSMB8F* and *PSMB8A* lineages. The F type *PSMB8* genes of Salmoniformes, Cypriniformes and sharks belong to the *PSMB8F* lineage, whereas all the other sequences belong to the *PSMB8A* lineage, showing that these two lineages have been established before the divergence of sharks from the bony vertebrates and that the *PSMB8F* lineage has been lost in higher teleosts and tetrapods. However, in *Xenopus* and higher actinopterygian teleosts (*Oryzias* and pufferfish), the new F type genes, possible functional equivalents of the *PSMB8F,* have revived as alleles within the *PSMB8A* lineage independently. To clarify the evolutionary history of the *PSMB8A* and *PSMB8F* lineage genes, we identified both of them in bichir, *Polypterus senegalus*, one of the most basal actinopterygian. Segregation analysis showed that the two lineage genes are alleles in bichir as well as in zebrafish and trout, whereas those of sharks are predicted to be paralogs behaving as pseudoalleles. These results suggested that the highly divergent allelic lineages of *PSMB8* have been retained for more than 400 million years in lower actinopterygians by an extremely long-term balancing selection. To clarify the genomic background of the PSMB8 dimorphism we also performed a physical analysis of the MHC region around the bichir *PSMB8* gene, using a bacterial artificial chromosome (BAC) clone. The order and orientation of the flanking genes of *PSMB8* of bichir was identical with that of *X. tropicalis* but quite different from those of higher teleosts, indicating that extensive genomic reorganization of the MHC region occurred in the teleost lineage after the divergence of bichir.

Evolution of Teleost MHC Class II Genes Revealed by Comprehensive Analysis of Medaka

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The Major Histocompatibility Complex (MHC) class II molecules play central roles in acquired immunity by regulationg immune response through activation of CD4 T cells. Each individual usually possesses one to a few classical MHC class II genes, which encode the MHC class II molecules, and some non-classical MHC class II genes, which encode other than MHC class II molecules. A full complement of the MHC class II genes has been elucidated only in mammalian species thus far. In mammals the lineage of the MHC class II genes have been conserved, although the data is incomplete and its evolution is still uncertain for non-mammalian vertebrates. To understand evolution of the MHC class II genes, we performed the first comprehensive analysis of the MHC class II genes in poikilothermic species using a teleost, medaka (*Oryzias latipes*). Through database search, cDNA cloning and genomic PCR, medaka was shown to possess five pairs of expressed class II genes, comprising one IIA and one IIB genes. Each pair is on a different chromosome and not linked to the class I genes. Only one pair showed a high degree of polymorphism and was considered to be classical class II genes, whereas the other four pairs were non-classical. Upon phylogenetic analysis of all medaka class II genes and most reported teleost class II genes, IIA and IIB genes formed separate clades, each containing three well-corresponding lineages. One lineage contained three medaka genes and all known classical class II genes of Ostariophysi and Euteleostei. The other two lineages contained one non-classical medaka gene each and some Euteleostei genes. These results indicate the presence of multiple lineages of the teleost MHC class II genes which have been conserved over three hundred million years.

Clonal culture of carp Th2-like cells whose immune status is hallmarked by IL-4/13B gene expression

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$CD4^+$ T helper (Th) cells play a central role in modulating vertebrate immune responses. In mammals, Th cells are subdivided into Th1, Th2, Th17 and Treg, according to their cytokine production profiles and expression of lineage-specific transcription factors. However in teleosts, many Th-related molecules have been identified but Th subsets have not been identified. Identification of Th subsets will help to understand the teleost immune system. Recently, we developed a bulk culture system in which co-culturing carp (*Cyprinus carpio*) leukocytes with supporting cells resulted in long-term proliferation of $CD4^+$ αβT cells. To characterize these bulk-cultured T cells by their cytokine production profile, we stimulated them with phytohaemagglutinin (PHA) and examined the expression of Th-related genes. Expression of both a Th1-related cytokine (*IFNγ*) and Th2-related cytokines (*IL-4/13A* and *IL-4/13B*) were enhanced by PHA stimulation. In addition, bulk-cultured T cells expressed transcripts of the Th1 and Th2 master regulators (*t-bet* and *gata3*, respectively). These results suggest that bulk-cultured T cells contained both Th1- and Th2-like cells. We attempted to identify these Th subsets by establishing and analyzing T cell clones. To establish T cell clones, single cells were picked up from bulk-cultured T cells, seeded onto the supporting cells and cultured. Cells proliferated in 1–4 wells of each 96-well plate. The majority of the clones expressed both *TCR-α* and *-β*, and also expressed either *TCR-γ* or *-δ*. Therefore these clones could produce the *TCRαβ* heterodimer but could not produce the *TCRγδ* heterodimer. These clones also expressed the TCR co-receptor gene *CD4-1*, but not *CD4-2*, *CD8α* or *CD8β*. In addition, *gata3* was expressed whereas *t-bet* was not. The gene expression profiles indicate that these clones also have characteristics consistent with $CD4^+$ αβT cell identity. One of these clones (KoThL5) continued to proliferate and was successively transferred for more than 10 months and 90 – 100 passages. In KoThL5 cells, PHA stimulation significantly enhanced the expression of *IL-4/13B*, but did not enhance the expression of *IFNγ* or another Th2-related cytokine, *IL-4/13A*. Together, these results indicate that KoThL5 cells represent a Th2-like subset which is hallmarked by enhanced expression of *IL-4/13B* and *gata3* expression.

Session: Vertebrate adaptive immune responce

Cell-mediated and humoral immune response to *Edwardsiella tarda* infection in ginbuna crucian carp, *Carassius auratus langsdorfii*

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Edwardsiella tarda is an intracellular pathogen, causes edwardsiellosis in fish. Cell-mediated immunity (CMI) has a major role in the protection against intracellular bacterial infection in mammals. In contrast the principal immune system against *E. tarda* in fish is still unclear. In order to clarify the importance of CMI and/or humoral immunity to prevent *E. tarda* infection in fish, we examined elimination of bacteria, CMI-related gene expression, population of leukocytes and production of antigen-specific antibodies in *E. tarda* infected ginbuna crucian carp. Ginbuna crucian carp (*Carassius auratus langsdorfii*) were infected with *E. tarda* at 10^5 CFU/100 g body weight (0.2 LD_{50}) by intraperitoneal injection and tissue (kidney, spleen and liver), kidney leukocytes (KLs) and plasma were collected 0, 2, 4, 8, 12, 16, 30 days post-infection. Bacterial counts in the tissues were determined by miles and misra methods. The expression analysis of CMI related genes such as IFNγ and perforin in KLs was performed by semi-quantitative RT-PCR. Proportion of $CD8\alpha^+$ and $sIgM^+$ cells in KLs were examined by influences assay with monoclonal antibody against ginbuna CD8α and IgM, respectively. *E. tarda*-specific antibody titer in plasma was determined by ELISA. Expression level of *IFNγ* and *perforin* were up-regulated from 2 days to 12 days post-infection. In addition proportion of $CD8\alpha^+$ cells was increased from 4 days to 8 days. These results suggested that CMI was induced in fish infected with *E. tarda*. The reduction of bacterial counts was observed from 2 days to 12 days, suggesting that CMI mainly contributed to elimination of *E. tarda* in the early stage of *E. tarda*-infection. On the other hand, *E. tarda*-specific antibody titer was increased after clearance of bacteria, indicating that protection against *E. tarda*-infection should be able to occur without humoral immunity. These findings suggested that CMI might be the principal immune system in the protection against *E. tarda* in fish.

Protection of ginbuna crucian carp (*Carassius auratus langsdorfii*) against *Edwardsiella tarda* infection using adoptive transfer of cytotoxic T lymphocytes

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Cell-mediated immunity (CMI) plays a major role in protection against intracellular pathogens in mammals. In particular, cytotoxic T lymphocytes (CTLs) play a crucial role in resolving intracellular infections. Activated CTLs induce effector molecule-mediated lysis of the infected cells: they secret cytokines such as interferon gamma (IFNγ) and induce CMI and Th1 polarization. Recently, we showed that CMI might be the principal component of the immune system in preventing infection from *E. tarda*, intracellular bacteria found in fish. In this study, to elucidate the contribution of CTLs in protection against *E. tarda* infection in fish, we used an adoptive transfer model established in clonal ginbuna crucian carp. We conducted a challenge test and analyzed CMI-related gene expression. $CD8\alpha^+$ leukocytes were isolated from the kidney of *E. tarda*-sensitized (10^5 CFU/100 g body weight [0.2 LD_{50}]) ginbuna crucian carp on day 8 after injection using magnetic cell sorting with anti-ginbuna CD8α monoclonal antibody. Donor cells (1.5×10^5 cells) were adoptively transferred to isogenic recipient fish via the caudal vein. The challenge test was performed with an LD_{100} of *E. tarda* by i.p. injection one day after transfer: all fish receiving sensitized $CD8\alpha^+$ cells survived, whereas some of the fish with non-sensitized $CD8\alpha^+$ cell transfer died and others showed serious clinical signs. This result indicated that CTLs play an important role in protection against *E. tarda* infection. IFNγ gene expression in the leukocytes of the head kidney, trunk kidney, spleen, and peripheral blood was analyzed in recipient fish on day 2 after challenge using quantitative PCR. The expression levels of *ifng1* and *ifng2* in the leukocytes of all the tissues were higher in the fish receiving sensitized $CD8\alpha^+$ cells than in the fish with non-sensitized $CD8\alpha^+$ cell transfer. Thus, sensitized CTLs act as a major cell type for IFNγ-production in *E. tarda*-infected fish. Moreover, these finding suggested that sensitized CTLs induce CMI during the early stages of *E. tarda* infection.

Three dimensional models of Signaling Lymphocyte Activation Molecules (SLAM), a receptor for morbillivirus in carnivores

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Morbillviruses belonging to the family Paramyxoviridae cause highly contagious diseases in humans and animals. To date, six virus species including measles virus (MV) and canine distemper virus (CDV), have been identified in land and marine mammals. A notable biological feature of morbillivirus is its high level of host specificity. Signaling Lymphocyte Activation Molecule (SLAM), belonging to immunoglobulin superfamily, is the principal cellular receptor for morbilliviruses allowing their entry and propagation in the host animals, and is a major determinant of the host specificity. The immunoglobulin-like V (IgV) domain in the extracellular region of SLAM, provides an interface to bind with morbillivirus H protein. The natural host for CDV is dogs, but the host range has been recently expanded to include other wild carnivorous animals. CDV caused more than 18,000 deaths of Baikal seals, and also induced fatal diseases to large felids such as lion or jaguars. To compare the interface to the virus on SLAM in the carnivores, we have determined the nucleotide sequences of the IgV domains of SLAMs from two Japanese wildcat species, Iriomote wildcat (*Prionailurus bengalensis iriomotensis*) and Tsushima leopard cat (*Prionailurus bengalensis euptilurus),* which are in danger of extinction. The sequences of the two wildcats were completely identical in the domain. We generated the 3D models on the base of the homology with proteins with known crystal structures: marmoset SLAM complexed with MV hemagglutinin and human NK, T, B cell antigens. We found thirty-two amino acid residues on the interface of SLAM IgV domain, which are potentially involved in the interaction with viruses. The two species of Japanese wildcats possessed several specific residues (S67, K72, T76, S82, and A87) among the thirty-two residues, and their interfaces showed a positively charged electrostatic surface compared to other carnivores. This result may indicate that the Japanese wildcats are less sensitive against CDV.

The Plasmablast-like Leukocyte in the Kidney of Fugu (*Takifugu rubripes*)

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The process of B cell differentiation into plasma cells, which are antibody secreting cells, has been largely unexplored in fish. Research on the differentiation and regulation into the plasma cells provides us with a better understanding of fish adaptive immunity. In this study, we isolated a unique leukocyte population from fugu (*Takifugu rubripes*) kidney, which is the major immune organ in teleost. This leukocyte population showed phenotypic properties similar to those of mammalian plasmablasts. First, the adherent cells from the fugu kidney expressed IgM protein on their surface. Flow cytometry showed that these cells were mainly composed of two populations: $IgM^+CD8\alpha^-$ cells and $IgM^+CD8\alpha^+$ cells. An reverse transcription PCR (RT-PCR) analysis demonstrated that the purified $IgM^+CD8\alpha^-$ cells expressed secretory-type IgM as well as Bcl-6 and Blimp-1, developmental marker genes for the B cell lineage, but not membrane-type IgM and Pax-5 genes. In addition, we examined the cytokine expression profile of the $IgM^+CD8\alpha^-$ cells by RT-PCR. Most of the cytokine genes were evidently not expressed by the $IgM^+CD8\alpha^-$ cells, whereas the $IgM^+CD8\alpha^+$ cells expressed various cytokines. Western blotting confirmed that the $IgM^+CD8\alpha^-$ cells secreted IgM protein. These results indicate that $IgM^+CD8\alpha^-$ cells are similar to the plasmablasts in mammals. This is the first report isolating plasmablast-like leukocytes in fish species. Our data also suggest that the teleost kidney is an organ where B cells terminally differentiate into the plasma cells.

Session: Vertebrate Innate/Homeostatic Response

Expression of Type-I Interferon Gene Controlled by IRF3/7 Mediated through TBK1 in Japanese Flounder

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Type I interferon (IFN-I) plays a very important role in the enhancement of antiviral innate immunity. In a previous study, the IFN-I promoter region in Japanese flounder (*Paralichthys olivaceus*), which initiates response to poly I:C, was identified and its transcriptional activity was shown to be dramatically enhanced by IFN regulatory factor (IRF) 3. In this study, we found new functional differences between IRF3 and IRF7 in the IFN-I induction pathway. The IFN-I transcriptional activity was significantly enhanced by IRF7 in HINAE (flounder natural embryo) cells when stimulated by poly I:C (a dsRNA mimic). To understand this function of IRF7, TANK-binding kinase 1 (TBK1, Serine/threonine protein kinase) cDNA was cloned from Japanese flounder. The deduced amino acid sequence of its domains was highly conserved with known vertebrates and it was shown to be weakly induced in the whole kidney and HINAE cells after stimulation by poly I:C and VHSV (viral hemorrhagic septicemia virus). In TBK1-overexpressing HINAE cells, IFN-I transcriptional activity was sufficiently enhanced and the expression of IFN-inducible gene (*i.e.* Mx and ISG15) was strongly induced. IFN-I activity was also induced by cotransfection with both TBK1 and IRF7, but not by cotransfection with IRF7 only. This evidence is very similar to the IRF7 activation by poly I:C described above. To confirm the relationship between IRFs and TBK1 in the IFN-I induction pathway, the transcriptional activities of IRFs were measured after the cells were treated with the TBK1-inhibitor, BX-795. Activity of IRF7 mediated by TBK1 or poly I:C was inhibited by BX-795 in a dose-dependent manner, but that of IRF3 was not. Furthermore, IFN-I activity mediated through TBK1 was enhanced by Toll-like receptor 3 (TLR3) cotransfection. These results suggested that the transcriptional regulation of Japanese flounder type I IFN gene is regulated by IRF3 and IRF7, but its regulation by IRF7 is controlled by TBK1 after TLR3 triggers the recognition of extrinsic dsRNA.

Poster Presentation

Session: Cytokine and chemokine

Earthworm extract possessed anti-inflammatory property through the down-regulation of IL-8 transcription

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Earthworm is a member of Oligochaeta in the phylum Annelida. It was well known as a bite for the mole, birds and others animals. Earthworm has been also recognized to use in oriental medicine. Previous reports demonstrated that earthworm extract revealed to exhibit cytolytic activities, proteolytic activities, anti-bacterial activities and anti-inflammatory property. However, precise mechanism of these pharmaceutical effects was not fully clarified yet. We have examined the effect of earthworm (*eisenia fetide*) extracts on the cytokine expression in human epithelial cell lines and mouse macrophages. Human epithelial cells, HT-29 and Ca9-22 cells originate from the alimentary tract and mouse peritoneal macrophages were used in this study. The cells, stimulated by TNF and lipopolysaccharide, were co-cultured in various concentrations and times with earthworm extracts. Cytokines, like IL-1, Il-6, IL-8 and TGF-β were assessed by the RT-PCR, real time PCR and Western blot analysis.

The extract clearly reduced IL-8 transcription level on the HT-29 and Ca9-22 cells following TNF stimulation. While, other cytokine transcription did not significantly change. Western blot analysis indicated that HT-29 and Ca9-22 cells decreased expression of the NF-kB, p38 and pErk1/2. In addition, earthworm extract reveled similar effect to the mouse peritoneal macrophages, which reduced the MIP-2 (functional IL-8 homologues in mouse) RNA transcription following stimulation with LPS. Our results suggested that the earthworm extract have the anti-inflammatory activity due to suppress the IL-8 secretion by MAPK inhibitory function.

Teleost IL-6 promotes antibody production through STAT3 signaling via IL-6R and gp130

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Antibodies play an important role in the adaptive immune system of teleost fish. When teleost fish encounter pathogens, they increase secretion of specific antibodies into the blood and external mucus. In mammals, antibody production is mainly regulated by interleukin-6, IL-6. Binding of IL-6 to the receptor complex consisting of IL-6 receptor (IL-6R) and glycoprotein-130 (gp130) stimulates intracellular signal transduction, that is, phosphorylation of STAT3. STAT3 activated by IL-6 then directly activates IgM-gene transcription. Teleost IL-6 is upregulated after antigen stimulation; therefore, we hypothesized that fish IL-6 contributes to antibody production during immune responses against infections. In this study, the membrane and soluble forms of IL-6R homologues cDNA were cloned in fugu (*Takifugu rubripes*). The cDNA of gp130 was also cloned. Three STAT3-docking sites were found in the intracellular region of fugu gp130. Fugu IL-6R and gp130 were expressed in the membrane IgM^+ ($mIgM^+$) B cells of leukocyte populations in the kidney and peripheral blood, suggesting that fugu B cells are stimulated by IL-6. Recombinant fugu IL-6 (rfIL-6) increased the gene expression of secretary antibodies by $mIgM^+$ B cells *in vitro*. Western blotting analyses showed that the rfIL-6 activated STAT3 phosphorylation in B cells and a cultured cell line cotransfected with fugu IL-6R and gp130. Multiple STAT-binding sites were found at the fugu immunoglobulin heavy chain gene locus. These results indicate that fugu IL-6 enhances antibody production in the B-cell lineage via gp130 and STAT3 signaling.

Session: Ig and other antigen receptors

Sequencing immunoglobulin variable regions of new antigen receptor genes in the banded houndshark, *Triakis scyllium*

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Cartilaginous fish possess the immunoglobulin isotype new antigen receptor (IgNAR), which is a heavy chain homodimer and does not associate with the light chain. The IgNAR genes are encoded in the cluster configuration, each of which contains one variable (V) segment, some diversity (D) segments, one joining (J) segment and a constant domain. It was reported that IgNAR variable regions have very high level of diversity in the complementarity determining region 3, where V, D and J segments are rearranged. We have cloned the cDNA encoding IgNAR from the banded houndshark (*Triakis scyllium*) and showed that this shark can produce a variety of IgNAR. Because of this, IgNAR can be a potential source of artificial single chain antibodies. In order to generate the single chain antibody efficiently, sequence information on the genes encoding IgNAR variable regions are needed, we therefore cloned IgNAR variable region from the banded houndshark. The genes encoding this region were amplified with specific primer sets designed using the cDNA sequences. The fragments were amplified from genomic DNA extracted from the muscle and subcloned into pGEM-T easy vector. We isolated and sequenced 27 clones. Twelve (12) clones were about 1.9kbp, and 2 clones were 1.4 kbp and the rest were 1.2 kbp long. Those showing the same length were identical, and thus were grouped into 3 individual sequences. The V and J segments of these clones were almost identical, while the D segments of the genes were distinct. It was also reported that the nurse shark (*Ginglymostoma cirratum*) and spiny dogfish (*Squalus acanthias*) have several IgNAR genes. Therefore, these results suggest that the banded houndshark might have at least 3 IgNAR genes. We are now evaluating the number of IgNAR genes by Southern blotting.

Session: Innate cellular immunity

Fatty acid binding protein 7 regulates the functions of murine macrophages

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Fatty acid binding protein (FABP) is the family of cytoplasmic proteins with molecular weight of 14-15 kDa, having a phylogenically reserved structure. FABP serves as vehicle of water insoluble fatty acids (FAs), and may consistently play important roles throughout animal evolution in the regulation of lipid metabolism, intracellular signaling mediated by FA metabolites, and gene expression by nuclear receptors which require fatty acids as ligand. As it is known that dietary polyunsaturated fatty acids affect the immune responses both in invertebrates and vertebrates, members of fatty acid binding protein superfamily have been studied with relation to innate immunity in both invertebrates and invertebrates. In mammal, FABP4 and 5 are generally expressed in macrophages except for Kupffer cells (KCs) of liver which specifically express FABP7. FABP4 and 5 have been shown to regulate the macrophage functions in adipose tissue and in the athelosclerotic plaque, but the role of FABP7 in KCs remained unknown. In this study, we explored the functions of FABP7, a member of the FABP family that preferentially binds to omega-3 PUFA, in KCs. In the mouse liver injury model induced by CCl_4 injection, FABP7 was detected in the F4/80+ macrophages before and after the treatment. FABP7 deficient (KO) mice showed the increase in the levels of serum ALT and AST, and infiltration of F4/80+ macrophages into damaged area was significantly decreased compared to wild-type. The phagocytic activity of KCs against intravenously injected apoptotic thymocytes (ATC) was significantly decreased in KO mice. Interestingly, serum level of TNF-alpha was decreased in KO mice in D-GalN/LPS induced mouse hepatitis models, and conversely, FABP7-overexpressing J774 cells showed increased activity of anti-apoptotic cell phagocytosis and of LPS-induced TNF-alpha production. Taken together, it was suggested that FABP7 is involved in the regulation of macrophage functions in mice.

Carp thrombopoietin in combination with kit ligand a induces the formation of colonies derived from unipotent progenitors for thrombocytes and bipotent progenitors for thrombocytes and erythrocytes

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In mammals, thrombopoietin (TPO) and Kit ligand (KitL; stem cell factor) are primary regulators of thrombopoiesis. TPO stimulates megakaryocyte progenitor cells (CFU-MK) and induces megakaryocyte colony formation *in vitro* in a semi-solid medium. Megakaryocyte colony formation is enhanced by TPO in combination with KitL, but not by KitL alone. To understand the non-mammalian thrombopoiesis, we produced recombinant carp TPO and kit ligand a (kitla), and developed a culture system capable of examining quantitatively and qualitatively analyzing of thrombocyte progenitors in carp. About 100,000 hematopoietic cells from carp kidney were cultured in a semi-solid methylcellulose medium. TPO induced colony formation in a dose-dependent manner. The maximum number of colonies was 110 - 150 per plate and all colonies were small (type 1), which were 50 to 100 μm in diameter. However, kitla alone did not induce colony formation. In addition, in the presence of both TPO and kitla, 300 - 380 colonies formed, the majority of which were type 1 and several larger colonies (200-500 μm in diameter) also formed. The larger colonies were divided into two types (types 2 and 3) based on their morphologies. Using RT-PCR, Type 1 cells were found to express a thrombocyte marker *c-mpl* (TPO receptor), type 2 cells were found to express both *c-mpl* and another thrombocyte marker *cd41* (a cell surface glycoprotein of thrombocyte lineage cells) and type 3 cells were found to express both of these markers and three erythrocyte markers (β*-globin*, *epo-r* and *gata1*). From these gene expression patterns, we considered that type 1 and type 2 colonies were derived from unipotent progenitors for thrombocytes. Type 2 colonies were derived from more immature progenitor cells than those of type 1 colonies. In contrast, type 3 colonies were derived from bipotent progenitors for thrombocytes and erythrocytes. In summary, TPO induced thrombocyte colony formation in a dose-dependent manner. The combination of TPO and kitla resulted in a synergistic enhancement of number of thrombocyte colonies, and induced the formation of mixed-type colonies derived from bipotent progenitors for thrombocytes and erythrocytes.

Session: Invertebrate hematocyte

Morphological Diversity of Crustacean Hemocytes

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Morphological characteristics of crustacean hemocytes were examined by light microscopy after staining with May-Grünwald stain using acid phosphate buffer (1/15 M, pH5.0) as a diluent. Only a single type of hemocyte (granulocyte) was observed in the primitive crustaceans like branchiopods (brine shrimp *Artemia salina* (A), fairy shrimp *Branchinellites kugenumaensis* (B), tadpole shrimp *Triops numidicus* (C), clam shrimp *Leptestheria kawauchieusis* (D), water flea *Daphnia pulex* (E)) and maxillopods (fish louse *Argulus japonicus* (F), seed shrimp *Vargula hilgendorfii* (G), ship barnacle *Lepas anatifera* (H), balanomorph barnacle *Megabalanus rosa* (I)). Staining characteristics of granules were different among these species. Two types of granules were observed in the hemocytes of A and G. The former had eosinophilic granules (EG) and chromophobic granules (CG), and the latter had basophilic granules (BG) and CG. In other species, the hemocyte had one type of granules (CG, excluding D (amphophilic granules)). The primitive malacostracan species, leaf shrimp *Nebalia japonensis* had only one type of hemocyte containing two types of granules (EG, BG). We call these species as monohemocytic crustaceans. On the other hand, plural hemocyte types were detected in the advanced malacostracans such as stomatopods (*Oratosquilla oratoria*, *Harpiosquilla harpax*), isopods (*Ligia exotica*, *Armadillidium vulgare*, *Asellus hilgendorfii*), amphipod (*Melita koreana*), and decapods. These species (polyhemocytic crustaceans) were classified into five groups (1-5) as below: 1, eight hemocyte types group (basophilic fine granular cell, basophilic plasma cell, basophilic granulocyte, chromophobic small granulocyte, chromophobic large granulocyte, basophilic and eosinophilic granulocyte, eosinophilic granulocyte (EGC; type 1 and 2)) including stomatopods, isopods (*L. exotica*, *A. vulgare*), amphipod, and decapods (Penaeidea, Thalassinidea, Brachyura); 2, four hemocyte types group (small basophilic cell, chromophobic granulocyte (CGC), eosinophilic small granulocyte (ESG), eosinophilic large granulocyte (ELG)) including decapods (Astacidea, Palinuridea, Anomura); 3, three hemocyte types group (CGC, ESG, ELG) including decapods (Astacidea, Brachyura); 4, two hemocyte types group (CGC, EGC) including isopod (*A. hilgendorfii*) and decapods (Stenopodidea, Caridea); 5, two hemocyte types group (ESG, ELG) including decapod (Brachyura). Based on these results, we make a speculation about the evolutional process of crustacean hemocytes.

Comparative morphology of hemocytes in deep-sea symbiotic bivalves, three *Bathymodiolus* mussels and two *Calyptogena* clams

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Hemocytes are involved in the defense system against microbial infections. Deep-sea bivalves are often dominant in deep-sea chemosynthetic ecosystems and mostly harbor methane-oxidizing and/or sulfur-oxidizing bacteria in their epithelial cells of gill tissues. Defense system probably plays important roles in the symbiotic interaction between the bacteria and the host animals. However, little is known about the defense system of deep-sea symbiotic bivalves. To understand the defense system in deep-sea symbiotic bivalves, we examined the hemocytes, which are thought to play a key role in the defense system, in five deep-sea symbiotic bivalves, *Bathymodiolus japonicus*, *B. platifrons*, *B. septemdierum*, *Calyptogena okutanii* and *C. phaseoliformis*, and classified them on morphological criteria. To see the functional difference among hemocytes, a phagocytosis assay using a FITC-labeled *Escherichia coli* was performed. Hemogram parameters (cells concentration, number and proportions of hemocyte types) and lectin-binding using four lectins (WGA, Con A, PHA-L, SBA) were also studied and compared in the bivalves. The results indicated that all of the bivalves have three types of hemocytes, a hemocyte type without granule and two types with granules. The two types were granular cell and hyaline cell. The hemocyte without granules was called agranular cell in *Bathymodiolus* mussels, and was smaller than the granular cell and hyaline cell. In two *Calyptogena* clams, this type of hemocyte was the red-blood cell having hemoglobin. In any of the examined bivalves, the granular cell showed the highest phagocytic activity and the resultant food vacuoles fused with lysosomes. The hyaline cell also showed the phagocytic activity with less extent but showed no fusion with the lysosome. The agranular cell and the red-blood cell showed no phagocytic activity. These results suggested that two types of granulocyte have the phagocytic activity which is the fundamental activity in the defense system against microbial infection in deep-sea symbiotic bivalves. The function of the agranular cell is still to be studied but the red-blood cell has hemoglobin, the oxygen carrier. Morphological study similarity suggested that the red-blood cell in Calyptogena clams is originated from the agranular cell.

Session: Invertebrate immune response

Slow transport of foreign objects to the distal end of the sea urchin larval arms

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The sea urchin and other echinoderm larvae have large advantages for studying developmental and comparative immunology, because the larva has a simple and transparent body consisting of small number of cells. The monumental findings using a kind of this larva are achieved by Iliya Metchnikoff. He found phagocytosis from the starfish larva when he injected a rose throne, a spine of a sea urchin and a glass tube. He also reported that the phagocytosis reaction consists merely in an accumulation of mesodermic phagocytes around the foreign body. However, he was not shown what happens after phagocytosing these undigested objects. If bacteria *E. coli* transformed by GFP-vector are injected into the blastocoel of a pluteus larva, GFP signal in the phagocytosed bacteria quickly get weak, showing the digestion of phagocytosed bacteria. In the case of undigested objects, a different defense system from digestion is expected to work, but it is still unveiled. In the present study, to find the next step of phagocytosis, we injected fluorescent latex beads and other objects with a micro-needle into the blastocoel of the sea urchin pluteus larva, and then tracked the beads during 5 days after injection. The beads were dispersed in the blastocoel just after injection, whose fluorescent signals were gradually reduced in the blastocoel. Instead, some of the beads were aligned on trunck spicules and arm spicules, which were prominent in the larvae 4 and 5 days after injection. The beads along with the spicules were migrated to the distal end of the arms revealed by an hour observation for each larva (n=10) under the microscope. Next, we injected FITC-labeled zymosan and RITC-labeled *E. coli* in the blastocoel of the larvae. A part of them are slowly transported to the distal end of the arms as well. It is still unknown where the foreign objects at the distal end go. We propose that the spicules of the sea urchin larva are functional for distancing foreign objects from the trunk containing a digestive organ and an adult rudiment to the distal end of the arms.

Characterization of a downstream component of Drosophila receptor guanylate cyclase activating innate immune response

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Drosophila receptor-type guanylate cyclases (rGCs) are novel immune related receptors identified from a genome-wide gain-of-function screening. rGCs are conserved in human as natriuretic peptide receptors producing cGMP in response to intrinsic ligands. Overexpression of rGCs induces *Drosomycin*, an antimicrobial peptide, through cGMP and downstream components of the Toll receptor, such as dMyD88, in a Toll receptor-independent manner in *Drosophila.* These results imply the existence of novel cGMP-mediated signaling pathway in immune activation. To elucidate its signaling mechanisms, we identifed a cGMP-dependent signaling molecule which acts downstream of rGCs in *Drosophila* larvae and characterized its function in *Drosophila* culture cells. Our results showed that the induction of RNAi targeting a cGMP-dependent protein kinase (cGK) completely abrogated rGC-induced *Drosomycin* expression in larvae. Overexpression of cGK and rGC strongly augmented the Toll-pathway-dependent *Drosomycin* expression in both larvae and *Drosophila* culture cells. Site-direct mutagenesis analysis showed that kinase-dead mutants of cGK failed to activate Toll-pathway. Furthermore, flies inducing RNAi targeting cGK were susceptible to infection of Gram-positive bacteria. These results suggest that *Drosophila* cGK is required for host defense and mediates the cGMP-dependent immune activation downstream of *Drosophila* rGCs.

Session: Pattern Recognition Molecules

Evolutional Conservation of Primordial Functions and Transcriptional Control of LGP2 Gene in Japanese Flounder, *Paralichthys olivaceus*

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LGP2 (laboratory of genetics and physiology 2), one of the pattern-recognition receptors, plays a very important role in antiviral response to induce production of type I interferon (IFN) through the recognition of cytosolic viral RNAs in the innate immune system. Although the expression of LGP2 mRNA in mammals and teleosts are strongly induced after virus infection or poly I:C stimulation, mechanism of transcriptional control of LGP2 gene is still unknown. Currently, LGP2 gene in Japanese flounder (*Paralichthys olivaceus*) was cloned. Japanese flounder LGP2 gene spanned 5,474 bp containing 12 exons and 11 introns. The expression of LGP2 mRNA dramatically increased in whole kidney of Japanese flounder infected with VHSV (viral hemorrhagic septicemia virus) 3 days post-infection and in whole kidney leukocytes stimulated with artificial double-stranded RNA (poly I:C) *in vitro*. Japanese flounder LGP2 has strong antiviral activities against VHSV, HIRRV (hirame rhabdovirus) or IPNV (infectious pancreatic necrosis virus) infected flounder natural embryo (HINAE) cell line. However when the RD (regulatory domain) of the LGP2 was deleted this function was lost. In addition, the expression of Mx and ISG15 in LGP2-overexpressed HINAE cells were strongly induced by poly I:C co-transfection but not by poly I:C-addition into the culture medium. The transcriptional control of Japanese flounder LGP2 gene was identified and its transcriptional activity was also analyzed by luciferase reporter assay. Numerous canonical motifs of IFN-regulatory factors (IRFs) were found in the 5'-upstream region (-1,337 bp) of LGP2 gene. Reporter assay showed that the poly I:C-responsive region regulating LGP2 transcription was identified at the location of -506 to -398. The transcriptional activity of poly I:C-responsive region was strongly enhanced by IRF3, which could bind to IRF3#3 motif located at -480, suggesting that LGP2 transcriptional control is probably involved in IRF3 function. Furthermore, LGP2 promoter was enhanced by VHSV infection in HINAE cells using GFP expression construct regulated by the LGP2 promoter including the poly I:C-responsive region. These results suggested that Japanese flounder LGP2 acts as a cytosolic viral RNA sensor, and the functions are conserved with those of mammalian LGP2 in the induction of antiviral response in the innate immune system.

Session: T-cell, MHC, and Ag-presentation

Different Evolutionary Patterns of Three Alpha Domains of the MHC Class *IA* Genes

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The major histocompatibility complex (MHC) genomic region contains many genes encoding various proteins, which play pivotal roles in the immune defense system of jawed vertebrates. MHC class I alpha chain gene (*IA* gene) is one of these and its product composes a class I molecule with beta-2 microgrobulin (B2M). MHC class I molecules function in the presentation of intracellular peptide antigens to cytotoxic $CD8^+$ T cells that kill those cells directly. Most vertebrates possess multiple *IA* loci and most of them are highly polymorphic possibly to be able to adapt to various kinds of antigens. Because genetic events such as gene duplication, deletion, recombination, and/or gene conversion have occurred frequently in these genes, the analysis of their evolutionary relationships between distantly related species has difficulties. We performed phylogenetic analyses using the nucleotide sequences of three alpha domains of the classical and non-classical *IA* genes independently, and found that these three domains have quite different evolutionary histories. In the analysis of the teleost fishes, the α1 domains generated four deeply diverged locus-specific lineages with high bootstrap values. Two of them contained the salmonid and medaka *IA* genes, unveiling the orthologous relationships between *IA* loci of salmonids and medaka, which diverged approximately 260 million years ago. In contrast, the α3 domains clustered by species or fish groups, regardless of classical or non-classical gene types, suggesting that this domain was homogenized in each species during prolonged evolution. On the other hand, the α2 domains formed no apparent clusters with the α1 lineages or with species, suggesting that they were diversified partly by interlocus gene conversion and that the α1 and α2 domains evolved differently. Although the primate *IA* genes evolved locus-specifically in all three domains, the homogenization of the α3 domain among classical and non-classical *IA* genes is also seen in some other vertebrates. Such distinct evolutionary patterns of three alpha domains might have contributed to the diversification of the peptide-binding site composed of the α1 and α2 domains, retaining the potential for CD8 binding as well as B2M binding in the α3 domain, during long-term evolution.

Characterization and expression analysis of CD4 and CD8 genes in yellowtail, *Seriola quinqueradiata*

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CD4 is a membrane glycoprotein that is mainly expressed on helper T (Th) cells and acts as a TCR coreceptor by binding to MHC class II molecules on antigen-presenting cells in mammals. Fish possess 2 types of CD4-like molecules, which are CD4-1 and CD4-2. The fish CD4-1 protein as well as the mammalian CD4 protein consists of 4 extracellular immunoglobulin (Ig)-like domains, a transmembrane domain and a cytoplasmic domain, whereas the fish CD4-2 proteins has only 2 or 3 Ig-like domains and a membrane proximal connecting peptide in the extracellular region. Mammalian CD8 is usually expressed on the surface of cytotoxic T lymphocytes (CTLs) as a heterodimer of α and β chains. In this study, we have cloned CD4-1 and CD8α homologues from the thymus of the yellowtail (*Seriola quinqueradiata*) by performing RACE. The yellowtail CD4-1 cDNA is 2122 bp long and encodes 467 amino acids. The deduced amino acid sequence of yellowtail CD4-1 had 4 extracellular Ig-like domains, a transmembrane domain, and a $p56^{lck}$-binding motif in the cytoplasmic domain, similar to the CD4-1 of other fish species and mammalian CD4. The yellowtail CD8α cDNA is 1649 bp long and encodes 217 amino acids. The deduced amino acid sequence of yellowtail CD8α cDNA was structurally similar to those of other vertebrates. Phylogenetic analysis showed that yellowtail CD4-1 and CD8α were clustered with other vertebrate CD4/CD4-1 and CD8α, respectively. Tissue-distribution analysis showed that yellowtail CD4-1 and CD8α as well as TCRα were predominantly expressed in lymphoid tissue, i.e., in the thymus, head kidney, and trunk kidney, and in mucosal tissue, i.e., the gill and intestine. In addition, the expression of these genes was detected in surface Ig-positive leukocytes but not in surface Ig-negative leukocytes. Moreover their expression levels were upregulated in the kidney of fish infected by *Mycobacterium* sp. at 2 months after infection. This result suggests that Th cells and CTLs play a role in protection against mycobacterial infection in the yellowtail.

Session: Vertebrate adaptive immune responce

Characterization of culture supernatant of *Streptococcus parauberis*: hemolytic factor and leucocyte-stimulation in Japanese flounder

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Two serotypes of *Streptococcus parauberis*, pathogens of streptococcosis in Japanese flounder (*Paralichthys olivaceus*), have caused severe losses of the livestock, due to the lack of effective vaccine and its resistance to antibiotics approved for flounder culture. These strains show different virulence, but their mechanisms of their pathogenicity are totally unknown, and identification of virulence factors are needed for effective vaccine development. The present study, therefore, was aimed at clarifying effect of *S. parauberis* culture supernatant on flounder. Since the diseased flounder shows anemia, we examined the effects of *S. parauberis* on flounder peripheral blood. As a result, *S. parauberis* -injected flounder showed decreased number of peripheral erythrocytes, suggesting that S. *parauberis* may produce a hemolytic factor. To characterize the hemolytic factor, a supernatant of *S. parauberis* culture (25˚C for 48h in Todd Hewitt broth) was mixed with sheep erythrocytes, resulting in significant level of hemolysis. The hemolytic factor was stable on heating at 100˚C for 10 min, and passed through an ultrafiltration membrane with a 5 kDa cut off limit (Amicon Ultra15), indicating that the factor is not proteinous hemolysin but a heat-resistant low molecular mass substance. We also investigated the effects of the culture supernatant on flounder leukocytes. Peripheral leukocytes were separated from flounder and carp using Percoll discontinuous density gradient centrifugation and cultured with the supernatant in 96-well plate. The stimulated flounder cells showed agglutination and significant proliferation as assayed by BrdU uptake, while carp cells did not. These results suggest that *S. parauberis* secrets a mitogen specific for flounder leucocytes. Characterization of the mitogen and resulting leucocyte response is in progress.

Session: Verterbate Innate/Homeostatic Response

Candidate key molecule(s) determining host specificity of parasite on fugu, *Takifugu rubripes*

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Fugu, *Takifugu rubripes*, is susceptible to infection with monogenean parasite, *Heterobothrium okamotoi*, which parasitize gills. Intriguingly, relative species of fugu, *Takifugu niphobles*, is not susceptible to the parasite although genetic distance between *T. rubripes* and *T. niphobles* is so close that their F_2 hybrids are viable. Genetic methods are applicable to *T. rubripes* for studying many biological aspects including host-parasite interactions since its genomic sequences have been analyzed and its comprehensive genetic map is also available. In the present study, we used F_2 individuals for genome-wide quantitative trait loci (QTL) analysis for parasite burden 21 days after challenge. As the result, we found a single major QTL peak on linkage group 9, and the region of the 95% confidential intervals contained 268 annotated genes. As this number of genes is still large, we have been conducting two additional experiments, i.e., cell surface display and subtractive PCR. By cell surface display, we successfully expressed two selected model membrane proteins on the surface of insect cells (High Five™). By using this platform, we are now screening candidates for receptors expressed in gills, which specifically bind to ligands on *H. okamotoi*. By subtractive PCR, we are trying to find genes, whose expression levels in gills are different between *T. rubripes* and *T. niphobles*. So far we found numbers of candidate genes including *pufflectin*. Pufflectin is a D-mannose specific lectin originally found in the skin mucus of fugu, and is expressed in skin as well as in gills. By semi-quantitative PCR and western blotting, we found that the expression of pufflectin is certainly much higher in *T. rubripes* than in *T. niphobles*. In our previous report, we showed that pufflectin could bind to body surface of *H. okamotoi*. These data suggests that pufflectin is one of the strong candidates for important factors determining host specificity of *H. okamotoi*. We are now conducting experiments to obtain direct evidences showing the relationship between the expression level of pufflectin and host specificity of *H. okamotoi*.

ISDCI 2012 Program Timetable (updated 2012 June 16)

Date								
July	9	Mon	11:00	Registration Starts			13:00 - 13:50	JADCI Business Meeting
			13:50 - 14:00	Welcome Address				
			14:00 - 15:00	Plenary Lecture by Prof. Shoichiro Kurata < Argos D >				
			15:00 - 15:30	Break				
				Room <Navis - C>		Room <Navis - B>		
			15:00 - 17:50	Cytokines and Chemokines - 1	15:30 - 18:00	Special Symposium "Recognition Mechanisms – Current Topics in Comparative Biology –"		
			18:30 - 20:00	Welcome Reception <Tinga-Tinga>				
			20:00 - 22:30	DCI Editorial Board meeting < Kusu >				
	10	Tue	9:00 - 10:00	Plenary Lecture by Dr. Zeev Pancer < Argos D >				
			10:00 - 10:30	Break				
				Room <Navis - C>		Room <Navis - B>		Room <Navis - A>
			10:30 - 11:30	Cytokines and Chemokines - 2	10:30 - 12:30	Pattern Recognition Molecules - 1	10:30 - 12:30	Ig and other antigen-receptors-1
			11:30 - 12:30	Antiviral Immunity - 1				
			12:30 - 13:30	Lunch < Argos E-F >				
			13:30 - 15:30	Antiviral Immunity - 2	13:30-14:30	Pattern Recognition Molecules - 2	13:30-15:30	Ig and other antigen-receptors-1
					14:30 - 15:30	Lectins - 1		
			15:30 - 16:00	Break				
			16:00 - 17:00	Antiviral Immunity - 3	16:00 - 16:40	Lectins - 2	16:00 - 17:00	Complement and Complement-like factors - 1
					16:40 - 17:20	Antimicrobial peptides/proteins - 1		
			17:30 - 20:30	Poster Session <Argos C >				
	11	Wed	8:45 - 9:45	Plenary Lecture by Dr. Claudia Kemper < Argos D >				
			9:45 - 10:15	Break				
				Room <Navis - C>		Room <Navis - B>		Room <Navis - A>
			10:15 - 12:15	Vaccine and Adjuvant	10:15 - 11:15	Antimicrobial peptides/proteins - 2	10:15 - 12:15	Complement and Complement-like factors - 2
					11:15 - 11:55	Innate Cellular Immunity - 1		
			12:15 - 13:15	Lunch < Argos E-F >				
			13:15 - 17:30	Excursion (Optional)	or	Free Afternoon		
			18:15 - 21:40	Dinner Cruise (Optional)				
	12	Thu	9:00 - 10:00	Plenary Lecture by Prof. Arturo Zychlinsky < Argos D >				
			10:00 - 10:30	Break				
				Room < Navis - C >		Room < Navis - B >		
			10:30 - 12:30	Vertebrate Adaptive Immune Response - 1	10:30 - 12:30	Innate Cellular Immunity - 2		
			12:30 - 13:30	Lunch < Argos E-F >				
			13:30 - 14:30	Vertebrate Adaptive Immune Response - 2	13:30 - 15:10	Invertebrate Hemocytes -1		
			14:30 - 15:10	Vertebrate Innate and Homeostatic Response - 1				
			15:10 - 15:40	Break				
			15:40 - 17:40	Vertebrate Innate and Homeostatic Response - 2	15:40 - 16:00	Invertebrate Hemocytes -2		
					16:00 - 17:00	Invertebrate Immune Response - 1		
			18:00 - 20:00	ISDCI Exec. Meeting				
	13	Fri	9:00 - 10:00	Plenary Lecture by Prof. Thomas Boehm < Argos D >				
			10:00 - 10:30	Break				
				Room <Navis - C>		Room <Navis - B>		
			10:30 -11:10	Vertebrate Innate and Homeostatic Response - 3	10:30 -12:30	Invertebrate Immune Response - 2		
			11:10 - 12:30	T cell, MHC, and antigen-presentation -1				
			12:30 - 13: 30	Lunch < Argos E-F >				
			13:30 - 14:50	T cell, MHC, and antigen-presentation -2	13:30 - 14:50	Invertebrate Immune Response - 3		
			14:50 - 15:20	Break				
			15:20 - 16:40	T cell, MHC, and antigen-presentation -3	15:20 - 16:20	Invertebrate Immune Response - 4		
			17:00 - 18:00	ISDCI General Meeting < Navis -BC >				
			19:30 - 22:30	Banquet < Argos E-F >				

Session allocation of abstracts
Updated 2012 June 19

* Each oral presentation will have 20 min (15 min talk & 5 min discussion).
* All the posters presentation will be held on Tuesday 10th.
* Presentations by JADCI members are indicated by ★

Session Name and Date	Day	Time	ID No.	Presenter	Title	
Special Symposium	Mon	15:30	SS-1	B-L. Lee	Recognition of Pathogenic or Symbiotic Bacteria and Immune Responses in Insects	★
		15:55	SS-2	R. Furukawa	Immunological Recognition in Starfish Larvae and Adults: The Alteration of Immune Cells Via Metamorphosis	★
		16:20	SS-3	D. Kojima	OPN5, a Photosensory Protein for Mammalian Ultraviolet Photoreception	★
		16:45	SS-4	H. Satake	Ligand recognition and signal transduction of GPCRs and Toll-like receptors of the protochordate, Ciona intestinalis: what is evolutionarily common or unique?	★
		17:10	SS-5	M. Yoshikuni	Comprehensive Analysis of Neuropeptides of Marine Invertebrates	★
		17:35	SS-6	T. Sakurai	Specificity and Sensitivity of Sex Pheromone Perception in the Silkmoth Bombyx mori	★

Session Name and Date	Day	Time	ID No.	Presenter	Title
Antimicrobial peptides/proteins (AMP)	Tue	16:40	AMP-O1	Xinhua Chen	Analysis on the gene diversity of hepcidin gene in large yellow croaker
		17:00	AMP-O2	James D. Pask	Normal constitutive secretion of skin peptides defends northern leopard frogs (Rana pipiens) against chytridiomycosis
		10:15	AMP-O3	Hai-Peng Liu	Antibacterial responses in the crab Scylla paramamosain
		10:35	AMP-O4	K. Wang	Scygonadin expression in Scylla paramamosain potentially associated with reproductive immunity
		10:55	AMP-O5	C. Li	Two novel families of antimicrobial peptides in the green sea urchin Strongylocentrotus droebachiensis
	Poster		AMP-P1	K. Shen	Escherichia coli DNA enhances the expression of attacin in Drosophila hemocyte cell line Mbn-2
			AMP-P2	M. Guo	Antiviral effects of beta-defensin derived from orange-spotted grouper, Epinephelus coioides
			AMP-P3	S. Wei	Molecular cloning and characterization of c-type lysozyme gene in orange-spotted grouper, Epinephelus coioides
			AMP-P4	S. Hipolito	Role of Marsupenaeus japonicus crustin-like peptide against Vibrio penaeicida and white spot virus infection
			AMP-P5	Bo-Hye Nam	An antimicrobial histone H1-like protein and its gene from the testes of Paralichthys olivaceus
			AMP-P6	C.H. Bigger	Antimicrobial activity in aqueous extracts of the caribbean octocoral Swiftia exserta.

Session Name and Date	Day	Time	ID No.	Presenter	Title	
Antiviral immunity (AVI)	Tue	11:30	AVI-O1	Chu-Fang Lo	Spawning stress triggers WSSV replication in brooders via the activation of shrimp STAT	
		11:50	AVI-O2	Apiruck Watthanasurorot	A gC1qR Prevents White Spot Syndrome Virus Replication in the Freshwater Crayfish Pacifastacus leniusculus	
		12:10	AVI-O3	Mei-An Su	The Litopenaeus vannamei LvRheb Protein is Involved in White Spot Syndrome Virus (WSSV) Pathogenesis	
		13:30	AVI-O4	S. Mangkalanan	Hemocytic Response in Persistent WSSV Infection in the Mud Crab, Scylla olivacea	
		13:50	AVI-O5	J-X Wang	Prohibitin interacts with VP28 and prevents WSSV replication in red swamp crayfish, Procambarus clarkii	
		14:10	AVI-O6	T. Somamoto	Functions of CD8-positive and CD4-positive lymphocytes against virus-infection in ginbuna crucain carp	★
		14:30	AVI-O7	P. H. Pham	Viral hemorrhagic septicemia virus genotype IV is differentially virulent toward Rainbow trout gills and monocyte/macrophage	
		14:50	AVI-O8	Fang-Huar Ngou	Virus-Host Interactions of Nervous Necrosis Virus in Grouper GK cell by Transcriptomic Analysis	
		15:10	AVI-O9	S. Patel	Nodavirus infection induces up-regulation of IFNgamma and T-helper cell markers.	
		16:00	AVI-O10	Joeri Kint	Mechanism of suppression of type-I interferon signaling by the chicken infectious bronchitis coronavirus	
		16:20	AVI-O11	B. Gorgoglione	ASSESSMENT OF THE IMMUNE RESPONSE MODULATION BY CO-INFECTIONS: PKD/VHS EFFECTS IN BROWN TROUT	
		16:40	AVI-O12	C. Langevin	Restriction mechanisms of fish rhabdoviruses by the zebrafish ISG15 protein	
	Poster		AVI-P1	C.-Y. Chang	Differential expression profiles of orange-spotted grouper under grouper iridovirus infection	
			AVI-P2	Y. J. Lee	Identification of the Envelope Protein VP51B of Shrimp White Spot Syndrome Virus (WSSV)	
			AVI-P3	T.Y. Chen	Grouper HSP90 Interacts with Nodavirus Coat Proteins and Participates in Nodavirus Replication	
			AVI-P4	T.Y. Chen	Identification of Leucine Zipper Domain of Antiviral Mx Protein Interfere with Nodavirus Coat Protein Localization	
			AVI-P5	H. Huang	The study of protein-protein interaction between white spot syndrome virus and glucose transporter Glut1	
			AVI-P6	Rong-yuan Chen	Identification of genes differentially expressed in in vitro haematopoietic tissue stem cells of Cherax quadricarinatus in response to WSSV infection	
			AVI-P7	Shau-Chi Chi	Betanodavirus-induced IFN response and Mx expression makes barramundi (Lates calcarifer) more resistant to red sea bream iridovirus infection	
			AVI-P8	Apiruck Watthanasurorot	New insights into extracellular function of TCTP as an antiviral infection in Pacifastacus leniusculus	
			AVI-P9	Youhua Huang	MAPK signaling pathway was involved in Singapore grouper iridovirus (SGIV) infection	

Session Name and Date	Day	Time	ID No.	Presenter	Title

Complement and Complemet-like factors (CMP)	Tue	16:00	CMP-O1	Irene Söderhäll	TEPs (thioester containing proteins) and ficolins; structure and function in a crustacean	
		16:20	CMP-O2	A. Tassanakajon	A Vital Role of Alpha-2-Macroglobulin in Prevention of Bacterial-Mediated Fibrinolysis in the Blood Coagulation System of Shrimp	
		16:40	CMP-O3	T. Sasaki	Cytolytic factor in the mosquito	★
	Wed	10:15	CMP-O4	T.Fujita	MBL-associated serine protease (MASP)-1/3 promotes the activation of the alternative complement pathway	
		10:35	CMP-O5	Vo Kha Tam	Isotypic Diversity in the Ontogenetic Expression of the Complement Component in the Common Carp (Cyprinus carpio)	★
		10:55	CMP-O6	Jun Li	Survivability of Edwardsiella tarda in Fish Serum Relates to Bacterial Surface LPS	
		11:15	CMP-O7	T. Yamaguchi	Identification of Pattern-Recognition Molecule in Hagfish Complement System	★
		11:35	CMP-O8	P. Kopáček	Functional Genomics of Tick Thioester-Containing Proteins and Other Complement-Related Molecules	
		11:55	CMP-O9	K. Tagawa	Microbe-specific C3b deposition in the horseshoe crab complement system in a C2/factor B-dependent or -independent manner	★
		Poster	CMP-P1	Rui Li	Unique Functions of MASPs in Amphioxus	
			CMP-P2	M. G. Castillo	Identification and Initial Characterization of a Thioester-Containing Protein (TEP) in the Squid Euprymna scolopes	

Session Name and Date	Day	Time	ID No.	Presenter	Title	
Cytokine and chemokine (CCK)	Mon	15:30	CCK-O1	D. Malagoli	Drosophila Helical factor acts as an inducible cytokine in S2 cells	
		15:50	CCK-O2	John Han-You Lin	The bioactivity of teleost IL-6: orange-spotted grouper (Epinephelus coioides) IL-6 induce Th2 cell differentiation pathway and antibody production	
		16:10	CCK-O3	Suhee Hong	Two types of TNFa in fish: cloning, expression and functional characterization of the third TNFa in rainbow trout	
		16:30	CCK-O4	H. Suetake	A chemokine expressed in the secondary lymphoid organs of fugu (Takifugu rubripes)	★
		16:50	CCK-O5	H. Korenaga	Identification, characterization and expression analysis of IL-17 receptors, Act1 and TRAF6 genes in Japanese pufferfish (Takifugu rubripes)	★
		17:10	CCK-O6	Y. Ding	The two interferons of Large yellow croaker	
		17:30	CCK-O7	Chris Secombes	MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF IL12 FAMILY MEMBERS IN SALMONIDS	
	Tue	10:30	CCK-O8	M. Chadzinska	Phylogenetic and functional diversity of CXC chemokines and their receptors in cyprinids	
		10:50	CCK-O9	C. Tafalla	Rainbow trout CK1 signals through CCR6 in B lymphocytes and is involved in the recruitment of IgT+ cells to mucosal sites	
		11:10	CCK-O10	C. Tafalla	Identification of a novel CCR7 gene in rainbow trout with differential regulation in the context of mucosal or systemic infection	
		Poster	CCK-P1	Linyi Hu	Characterization of TNF and TNF receptor superfamily in amphioxus	
			CCK-P2	T. Zheng	The interferon regulatory factor (IRF) family in amphioxus	
			CCK-P3	Ming-Wei Lu	The Functional Characterization of Zebrafish Type I Interferon (zfIFN) in Nervous Necrosis Virus	
			CCK-P4	G. Biswas	A Multiplex RT-PCR Assay for Rapid and Sensitive Detection of Cytokines in Pufferfish	
			CCK-P5	T. Iwase	Earthworm extract possessed anti-inflammatory property through the down-regulation of IL-8 transcription	★
			CCK-P6	O. Kurata	N-Terminal Region Responsible for Chemotactic Activity of Flounder IL-8	
			CCK-P7	M. Kaneda	Teleost IL-6 promotes antibody production through STAT3 signaling via IL-6R and gp130	★
			CCK-P8	Jui-Ling Tsai	Molecular cloning and characterization of orange-spotted grouper (Epinephelus coioides) IL-12	
			CCK-P9	M. Inada	Gene Knockdown and mRNA Variants of Astakine, Ancient Cytokine, in Kuruma Shrimp Marsupenaeus japonicus	
			CCK-P10	Chris Secombes	The interferon (IFN) system of salmonids is more complex than previously realised: Characterisation of the IFN locus in rainbow trout reveals two novel IFN subgroups	
			CCK-P11	Zhengliang Ou-yang	Molecular Cloning, Characterization and Expression Analysis of STING from Orange-spotted Grouper, Epinephelus coioides	
			CCK-P12	Chris Secombes	CLONING AND EXPRESSION ANALYSIS OF A THIRD IL1beta GENE IN SALMONIDS: TWO TYPES OF IL1beta GENES EXIST IN TELEOST FISH	

Session Name and Date	Day	Time	ID No.	Presenter	Title	
Ig and other antigen receptors (IGR)	Tue	10:30	IGR-O1	Y. Sutoh	VLRC defines a population of lamprey lymphocytes distinct from that expressing VLRA or VLRB	★
		10:50	IGR-O2	S. S. Higgie	The origin and evolution of the immunoglobulin domain	
		11:10	IGR-O3	H. Cortes	Understanding Channel Catfish (Ictalurus punctatus) leukocyte immune-type receptors (IpLITRs)	
		11:30	IGR-O4	B.C. Montgomery	Channel catfish leukocyte immune-type receptors mediate inhibition of cellular cytotoxicity by SHP-1-dependent and -independent mechanisms.	
		11:50	IGR-O5	J. Oriol Sunyer	Dominant role of IgT and IgT+ B cells in Rainbow Trout (Oncorhynchus mykiss) cutaneous immunity	
		12:10	IGR-O6	Katherine A. Rego	Characterization of a new immunoglobulin light chain isotype, Ig lambda, in rainbow trout, Oncorhynchus mykiss	
		13:30	IGR-O7	Ivar Hordvik	Characterization of IgM sub-variants in salmonid fish	
		13:50	IGR-O8	I. Salinas	Analysis of immunoglobulin heavy chain genes and proteins in the African lungfish Protopterus dolloi	
		14:10	IGR-O9	Nil R. Saha	Genomic survey of the immune repertoire in the living coelacanth	
		14:30	IGR-O10	Gang Cheng	Extensive diversification of IgH subclass encoding genes and IgM to IgM class switching in Crocodylia	
		14:50	IGR-O11	S. D. Fugmann	Separation of mutational and transcriptional enhancers in the Gallus gallus IGL gene	

		15:10	IGR-O12	P. Boudinot	Teleost fish mount complex clonal IgM and IgT responses in spleen upon systemic viral infection	
		Poster	IGR-P1	M.-S. Wu	Expression patterns of the immunoglobulin light chain genes in Epinephelus coioides	
			IGR-P2	S. Suda	Sequencing immunoglobulin variable regions of new antigen receptor genes in the banded houndshark, Triakis scyllium	★
			IGR-P3	Young-Kyu Kim	Characterization of Monoclonal Antibody Against Immunoglobulin Kappa Light Chain in Olive Flounder, Paralichthys olivaceus	
			IGR-P4	F. Ramirez-Gomez	Understanding secretory IgD in Rainbow trout: Antigen-specific plasma cell distribution and expression in body secretions	
			IGR-P5	Priti Kataria	The structure of rainbow trout (Oncorhynchus mykiss) IgM influences antibody-mediated phagocytosis	
			IGR-P6	Tao Wang	Evidence of IgY subclass diversification in the reptilian snakes: evolutionary implications 1, 2	
			IGR-P7	Priti Kataria	Competitive displacement of antibody secreting cells in rainbow trout	

Session Name and Date	Day	Time	ID No.	Presenter	Title	
Innate cellular immunity (INC)	Wed	11:15	INC-O1	E. Koppang	A role of melanin production in melanomacrophages?	
		11:35	INC-O3	Tomáš Korytář	Functional and Molecular Aspects of the Immune Defence in Peritoneal Cavity of Rainbow Trout (Oncorhynchus mykiss)	
	Thu	10:30	INC-O4	L. Pijanowski	Neutrophil extracellular traps in carp	
		10:50	INC-O7	Valerie J. Smith	Extracellular chromatin traps are an ancient eukaryotic cellular defence strategy	
		11:10	INC-O6	Elisabeth A. Dyrynda	Revisiting the cell: Differences in haemocyte death patterns associated with immunity in an invertebrate model	
		11:30	INC-O2	M. A. Kepka	Melatonin reduces ROS-dependent apoptosis of carp phagocytes	
		11:50	INC-O5	T. Nagasawa	Phagocytosis and bactericidal abilities of teleost thrombocytes.	★
		12:10	INC-O3	Daniel R. Barreda	Evolving Roles of Phagocytes for the Control of Inflammation	
		Poster	INC-P1	Julie Ghosh	The axial organ: the source of coelomocytes in the purple sea urchin?	
			INC-P2	H. Miyazaki	Fatty acid binding protein 7 regulates the functions of murine macrophages.	★
			INC-P3	Y. Sugie	Carp thrombopoietin in combination with kit ligand a induces the formation of colonies derived from unipotent progenitors for thrombocytes and bipotent progenitors for thrombocytes and erythrocytes	★
			INC-P4	M. Tomana	Flow cytometric characterization of Japanese bullhead and whale shark peripheral blood leukocytes	
			INC-P5	Yong-An Zhang	The Neural Cell Adhesion Molecules in Mandarin Fish	

Session Name and Date	Day	Time	ID No.	Presenter	Title	
Invertebrate hemocytes (IVH)	Thu	13:30	IVH-O1	V. Skara	Activity of Radix lagotis (Lymnaeidae) hemocytes: influence of infection by the bird schistosome Trichobilharzia regenti	
		13:50	IVH-O2	B. D. Parsons	Requirement of Pvr Ligands in Drosophila Embryonic Macrophage Survival and Migration	
		14:10	IVH-O3	P. Prochazkova	The regulation of cellular iron homeostasis in Eisenia andrei earthworms	
		14:30	IVH-O4	C. Noonin	CRAYFISH HEMATOPOIETIC TISSUE AS A MODEL FOR STEM CELL	
		14:50	IVH-O5	Jiann-Horng Leu	Identification of hematopoiesis-related genes in Litopenaeus vannamei	
		15:40	IVH-O6	Christine Paillard	Cellular and molecular analysis of Ruditapes philippinarum hemocytes reveals cytoskeleton disruption after Vibrio tapetis challenge	
		Poster	IVH-P1	A. Accorsi	Time-course analysis of hemocyte populations in the freshwater snail Pomacea canaliculata (Gastropoda, Ampullariidae)	
			IVH-P2	J. Y. Hwang	Flow cytometry analysis hemocytes of immune response of invertebrate due to infections	
			IVH-P3	M. Kondo	Morphological Diversity of Crustacean Hemocytes	★
			IVH-P4	D. Sekine	Monoclonal antibodies recognizing hemocytes and cells in gill tissue of deep-sea mussel, Bathymodiolus japonicus	
			IVH-P5	A. Tame	Comparative morphology of hemocytes in deep-sea symbiotic bivalves, three Bathymodiolus mussels and two Calyptogena clams	★
			IVH-P6	Irene Söderhäll	Crustacean hematopoietic factor - a regulator of hemocyte homeostasis	
			IVH-P7	A. Watthanasurorot	Hemocyte homeostasis the role of astakines	
			IVH-P8	Valerie J. Smith	Characterisation of prohaemocytes from crab, Carcinus maenas, and their survival in vitro	

Session Name and Date	Day	Time	ID No.	Presenter	Title
Invertebrate immune response (INV)	Thu	16:00	INV-O1	Christine Paillard	Nacrezation process, an immune defense system efficient against vibrios in manila clams?
		16:20	INV-O2	C. Le Bris	Demonstration of the increase of laccase-like activity in immune defense of clams infected by pathogens
		16:40	INV-O3	K. Somboonwiwat	Antibacterial and Antiviral Activity of Penaeus monodon Antilipopolysaccaride Factor Isoform 3 (ALFPm3)
		17:00	INV-O4	Lijun Feng	Identification and Characterization of an Amphioxus Matrix Metalloproteinase Homolog BbMMPL2 Responding to Bacteria Challenge
		17:20	INV-O5	R. Bettencourt	The emergence of the mussel Bathymodiolus azoricus as a bona fide model to study innate immunity in deep-sea vent animals
	Fri	10:30	INV-O6	Martin Bilej	Microbial environment determines the innate immunity in earthworms
		10:50	INV-O7	Han-Ching Wang	The diversified IgSF molecule, Dscam, and highly specific innate immune responses in shrimp
		11:10	INV-O8	Kenneth Söderhäll	Dscam (Down syndrome adhesion molecule); structure and function in a crustacean.
		11:30	INV-O9	Tze Hann Ng	Investigating Crustacean Immune Response via Putative Antigen-Specific Receptor, Dscam
		11:50	INV-O10	Patrick C. Hanington	FREP3 is an important factor in snail resistance to trematode infection
		12:10	INV-O11	Judith E. Humphries	Gene regulation of immune responses in Biomphalaria glabrata

		13:30	INV-O12	C. H. Bigger	Adaptive Immunity in a Coral: a Question of Memory?	
		13:50	INV-O13	S. Guntermann	Functional Analysis of the Fly Caspase Dredd in the IMD Innate Immune Signaling Pathway.	
		14:10	INV-O14	T. Shibata	Transglutaminase-catalyzed Relish Crosslinking Suppresses Innate Immune Signaling in the Drosophila Gut	★
		14:30	INV-O15	L. Zhang	Evolutionary and Regulation Dynamics of Immune-Related Genes and Pathways in the Pacific Oyster	
		15:20	INV-O16	Edan Foley	The Drosophila Pvr Pathway is an Intrinsic Autocrine Regulator of Intestinal Homeostasis.	
		15:40	INV-O17	C.J. Coates	Investigating Amebocyte Phagocytosis and Immune Defence in Limulus polyphemus	
		16:00	INV-O18	Moon-Soo Heo	Immunomodulation of green marine alga (Ulva lactuca) against Aeromonas hydrophila in Macrobrachium nobilii	
		Poster	INV-P1	J. Peng	Novel TIR adaptors acts as a negative regulator in inflammatory signaling of amphioxus	
			INV-P2	X. Dong	Ubiquitination regulatory of immune signaling pathway in amphioxus	
			INV-P3	M. Tsau	Cloning and analysis of autophagy-related protein 8 (ATG8) in shrimp Litopenaeus vannamei	
			INV-P4	F.Y. Chen	Gene cloning gene protein expression profiling and antioxidant activity of some immune associated parameters in Scylla paramamosain with LPS challenge	
			INV-P5	K Qiao	Gene cloning and expression analysis of a sigma class glutathione S transferase and molluscan caspase in haemocytes of variously colored abalone upon bacterial challenge	
			INV-P6	S.L. Hsieh	Effects of Dietary Gynura bicolor Extract on Immune Responses in White Shrimp, Litopenaeus vannamei	
			INV-P7	T. Itami	Molecular Cloning and Analysis of Toll Interacting Protein Gene, MjTollip, in Kuruma Shrimp Marsupenaeus japonicus	
			INV-P8	P. Jiravanichpaisal	Properties of an Interleukin-1 Receptor-Associated Kinase-4 (IRAK-4) of Black Tiger Shrimp (Penaeus monodon)	
			INV-P9	Jiann-Chu Chen	Modulation of the innate immune response in white shrimp following long-term low-salinity exposure	
			INV-P10	M. Inada	Molecular Cloning and Expression Analyses of Free Radical Generation Enzymes in Kuruma Shrimp Marsupenaeus japonicus	
			INV-P11	Tohru Mekata	Changes in transcription profiles of immune related genes after silencing of Toll receptors in shrimp	
			INV-P12	Taku Hibino	Slow transport of foreign objects to the distal end of the sea urchin larval arms	★
			INV-P13	Chadanat Noonin	Identification and functional study of thymosins in Pacifastacus leniusculus	
			INV-P14	Francisco Ramirez-Gomez	Highlights from cellular and molecular studies of the immune system of a Caribbean Sea cucumber	
			INV-P15	Hiroki Inoue	Characterizations of Novel Cadherins and Intercellular Recognition Molecules Related to Parasite-Host Compatible Cell Interactions.	
			INV-P16	Christopher J. Bayne	Resistance to Schistosoma mansoni is correlated with the numbers of pseudopod-producing hemocytes in Biomphalaria glabrata	
			INV-P17	S. Dharaneedharan	Magnifera indica kernel feed on immune response of Penaeus indicus against white spot syndrome virus (WSSV)	
			INV-P18	T. Itami	Interaction between WSSV infection and Apoptosis-, JAK/STAT-signaling- pathway- and RNAi-related Genes in Kuruma Shrimp, Marsupenaeus japonicus	
			INV-P19	T. Itami	Identification and Expression Analysis of Cytochrome c in Kuruma Shrimp, Marsupenaeus japonicus	
			INV-P20	Xin Tao	Amphioxus IKK complex involved in TLR modulated NF-kappaB and IRF activation	
			INV-P21	H. Kanoh	Characterization of a downstream component of Drosophila receptor guanylate cyclase activating innate immune response	★
			INV-P22	Yong-Chin Lin	The Antioxidant Role of Superoxide Dismutase in Penaeid Shrimp	

Session Name and Date	Day	Time	ID No.	Presenter	Title
Lectin (LEC)	Tue	14:30	LEC-O1	P. Angthong	Characterization of the Tachylectin-type Lectin in the Black Tiger Shrimp, Penaeus monodon
		14:50	LEC-O2	Laura F. Grice	Seeking diversity: Genomic characterisation of the aggregation factor genes of the demosponge *Amphimedon queenslandica*
		15:10	LEC-O3	Lijun Feng	Identification of an amphioxus intelectin homologue that preferably agglutinates gram positive over negative bacteria likely due to different binding capacity to LPS and PGN
		16:00	LEC-O4	Maria Forlenza	Analysis of Highly Expressed Short Immune-Related Soluble C-Type Lectin (SISC) in Common Carp (Cyprinus carpio)
		16:20	LEC-O5	A.M. Argayosa	Warm-water Fish Lectins and Their Relevance to Innate Immunity and Aquaculture Applications
		Poster	LEC-P1	Y. Ikazaki	Possible roles of Xenopus laevis XCGL family lectins in innate immunity
			LEC-P2	N. Wakamiya	The Biological Functions of the Novel Collectins CL-L1, CL-K1, and CL-P1
			LEC-P3	Y. Endo	Orthology between human and mouse ficolins, and roles of mouse ficolins A and B in host defense

Session Name and Date	Day	Time	ID No.	Presenter	Title
Pattern recognition molecules (PRM)	Tue	10:30	PRM-O1	S.V Nair	Variations to the sea urchin 185/333 genes during gametogenesis and ontogeny
		10:50	PRM-O2	M. O Roth	Somatic gene diversification in the sea urchin, Heliocidaris erythrogramma
		11:10	PRM-O3	C. M. Lun	A recombinant Sp185/333 immune protein from the California purple sea urchin shows specific and non-reversible binding to certain microbes
		11:30	PRM-O4	T.P. Yoshino	Innate immunity in snails Biomphalaria glabrata to schistosome infections: early recognition based on lectin-glycan interactions
		11:50	PRM-O5	Nil R. Saha	Extreme Functional Evolutionary Divergence of an Immune Receptor in a Basal Vertebrate
		12:10	PRM-O6	Danilo Pietretti	POSSIBLE ROLE IN RECOGNITION OF PROTOZOAN PARASITES: CHARACTERIZATION OF TLR20 IN COMMON CARP (CYPRINUS CARPIO)

		13:30	PRM-O7	Pinwen P. Chiou	Regulation of Grouper Toll-like Receptor 9 Signaling by Alternative RNA Splicing	
		13:50	PRM-O8	C.L. Yao	Molecular characterizations of two types of TLR5 in large yellow croaker, Larimichthys crocea	
		14:10	PRM-O9	Inge R. Fink	Studies into the Formation of Heterodimers of Toll-Like Receptor-1 and -2 in Common Carp	
		Poster	PRM-P1	Ming-Ching Lin	The shrimp anti-lipopolysaccharide factor (SALF) inhibits the expression of proinflammatory cytokines by *Trichomonas vaginalis* -infected HeLa cells.	
			PRM-P2	M.X. Chang	Identification of peptidoglycan recognition protein 6 and its splicing isoforms that inhibit intracellular bacterial proliferation in grass carp CIK cells	
			PRM-P3	H. Chou	Does opsonization of bacteria by a recombinant Sp185/333 protein from the California purple sea urchin augment phagocytosis?	
			PRM-P4	Lauren S. Sherman	Diversity of immune response proteins: nickel-isolated Sp185/333 proteins from the purple sea urchin	
			PRM-P5	T. Aoki	Evolutional Conservation of Primordial Functions and Transcriptional Control of LGP2 Gene in Japanese Flounder, Paralichthys olivaceus	★
			PRM-P6	Danilo Pietretti	CHARACTERIZATION OF TOLL-LIKE RECEPTOR 4 IN COMMON CARP (CYPRINUS CARPIO)	
			PRM-P7	Danilo Pietretti	Identification of several CRP-like sequences in common carp increases the complexity of the pentraxin system	
			PRM-P8	Seong Don Hwang	Molecular identification and expression analysis of two distinct BPI/LBPs from rock bream, Oplegnathus fasciatus	
			PRM-P9	R. Harikrishnan	Identification and molecular characterization and mRNA transcription in tissues of the apolipoprotein E in Epinephelus bruneus	
			PRM-P10	SeongDon Hwang	Molecular cloning and functional characterization of Toll-like receptor 3 in Japanese flounder, Paralichthys olivaceus	

Session Name and Date	Day	Time	ID No.	Presenter	Title	
T-cell, MHC, and Ag-presentation (TCM)	Fri	11:10	TCM-O1	N.T Fujito	Long-term Transspecies Dimorphism of the PSMB8 Gene in the Actinopterygian MHC Region	★
		11:30	TCM-O2	C. Huang	Recurrent Revival of Dimorphism of the PSMB8 Gene During Tetrapod Evolution	
		11:50	TCM-O3	H. Bannai	Evolution of Teleost MHC Class II Genes Revealed by Comprehensive Analysis of Medaka	★
		12:10	TCM-O4	Uwe Fischer	Phenotypic differences of mucosal and non-mucosal CD8alpha positive T-cells in the teleost Oncorhynchus mykiss	
		13:30	TCM-O5	M.F. Criscitiello	Somatic hypermutation for primary alpha/beta T cell repertoire generation in shark	
		13:50	TCM-O6	G. Kato	T cell marker CD4 and CD8 gene in Japanese flounder, Paralichthys olivaceus	
		14:10	TCM-O7	G. Scapigliati	T cells of the teleost sea bass *Dicentrarchus labrax* (L.)	
		14:30	TCM-O8	Huong Dang Thi	A profile of peripheral and mucosal T-cell subpopulations in rainbow trout (Oncorhynchus mykiss)	
		15:20	TCM-O9	T. Yamaguchi	Clonal culture of carp Th2-like cells whose immune status is hallmarked by IL-4/13B gene expression	★
		15:40	TCM-O10	Yuko Ota	Evolution of the B7 family coevolution and the B7s historical relationship with the MHC	
		16:00	TCM-O11	David Parra	Phagocytic B cells discovered in mammals: Murine B-1 B cells have phagocytic and microbicidal capacities and present phagocytosed antigen to CD4+ T cells	
		16:20	TCM-O12	Fumio Takizawa	First identification of regulatory B cell subsets expressing IL-10 in teleost fish	
		Poster	TCM-P1	S. Yoon	CHARACTERIZING THE ROLE OF CD4+ CELLS WITHIN THE ZEBRAFISH (DANIO RERIO) IMMUNE RESPONSE	
			TCM-P2	P. Johansson	Characterising putative dendritic cell markers in rainbow trout (Oncorhynchus mykiss)	
			TCM-P3	Y. Yan	Functional studies on an immune-related gene of MyD88 in orange-spotted grouper, Epinephelus coioides	
			TCM-P4	M. I. Nonaka	Different Evolutionary Patterns of Three Alpha Domains of the MHC Class IA Genes	★
			TCM-P5	R. Nagamine	Characterization and expression analysis of the Japanese pufferfish Takifugu rubripes homologue of the mammalian Th-POK	
			TCM-P6	K. Araki	Characterization and expression analysis of CD4 and CD8 genes in yellowtail, Seriola quinqueradiata	★
			TCM-P7	Jorunn B. Jørgensen	CD40L An important Costimulatory molecule involved in the maturation of Antigen Presenting Cells in Atlantic salmon.	
			TCM-P8	Chun Xia	Narrow Groove of MHC Class I Molecule BF2*0401 Plus Peptide Transporter Restriction can Explain Disease Susceptibility of B4 Chickens	
			TCM-P9	Fumio Takizawa	Identification and transcription analysis of Eomesodemin-a and -b in rainbow trout	

Session Name and Date	Day	Time	ID No.	Presenter	Title
Vaccine and adjuvant (VAC)	Wed	10:15	VAC-O1	H. L. Thim	INCREASED PROTECTIVE IMMUNE RESPONSES WHEN COMBINING TLR-AGONISTS IN A WHOLE-VIRUS ANTIGEN VACCINE AGAINST SALMONID ALPHAVIRUS
		10:35	VAC-O2	K. Villumsen	Protective effects of vaccine-induced specific antibodies against Aeromonas salmonicida subsp. salmonicida in rainbow trout
		10:55	VAC-O3	L. Neumann	Oral and anal vaccination against enteric red mouth disease induces full protection against yersiniosis.
		11:15	VAC-O4	M. K. Raida	Immersion vaccination against Yersinia ruckeri O1, biotype 2 confers cross protection against Y. ruckeri O1 biotype 1.
		11:35	VAC-O5	L. G. Jørgensen	Improved ERM vaccination efficacy using combined vaccine administration methods
		11:55	VAC-O6	Dimitar Iliev	CpG activation of distinct MHCII+ cell types in Atlantic salmon (Salmo salar)
		Poster	VAC-P1	Dong-Hwi Kim	Effects of chitin and chitosan particles on innate immune system in Epinephelus bruneus
			VAC-P2	M.-G. Kwon	The efficacy and safety of oil-adjuvanted combination vaccine containing Edwardsiella tarda, Streptococcus iniae and Streptococcus parauberis in olive flounder Paralichthys olivaceus
			VAC-P3	A. Taechavasonyoo	The immuno-adjuvant effect of interleukin-1 beta in Japanese flounder (Paralichthys olivaceus)

			VAC-P4	S. Chen	Vaccine efficacy of recombinant proteins from Nocardia seriolae against nocardiosis in large mouth bass, Micropterus salmoides
			VAC-P5	Bo-Wei Chi	Development of oral delivery system for aquaculture viral disease using Listeria monocytogenes

Session Name and Date	Day	Time	ID No.	Presenter	Title	
Vertebrate adaptive immune response (ADP)	Thu	10:30	ADP-O1	Makoto Inami	Intestinal immunity in cod	
		10:50	ADP-O2	M. K. Raida	Association between increased antibody level and protection in Yersinia ruckeri bacterin immersion vaccinated rainbow trout.	
		11:10	ADP-O3	M. Yamasaki	Cell-mediated and humoral immune response to Edwardsiella tarda in ginbuna crucian carp, Carassius auratus langsdorfii	★
		11:30	ADP-O4	K. Araki	Protection of ginbuna crucian carp (Carassius auratus langsdorfii) against Edwardsiella tarda infection using adoptive transfer of cytotoxic T lymphocytes	★
		11:50	ADP-O5	K. Ohishi	Three dimensional models of Signaling Lymphocyte Activation Molecules (SLAM), a receptor for morbillivirus in carnivores	★
		12:10	ADP-O6	M. Edwards	Immune protection of developing marsupial pouch young: what does transcriptome sequencing tell us?	
		13:30	ADP-O7	L.Jørgensen	Immunity against the parasite Ichthyophthirius multifiliis in rainbow trout	
		13:50	ADP-O8	Louise A. Rollins-Smith	Deadly chytrid fungus can paralyze amphibian lymphocyte responses	
		14:10	ADP-O9	T. Odaka	The Plasmablast-like Leukocyte in the Kidney of Fugu (Takifugu rubripes)	★
		Poster	ADP-P1	Seung-Hyun Hong	Dietary Rubus coreanus on immune system in Epinephelus bruneus against Vibrio alginolyticus	
			ADP-P2	Ronggai Li	B-cell markers and cytokines involved in B-cell growth and differentiation from cartilaginous fish	
			ADP-P3	S. Boltaña	Immune response in sea bream (Sparus aurata) after treatment with LPS of Aeromonas salmonicida and Listonella anguillarum	
			ADP-P4	B. Gorgoglione	MOLECULAR CHARACTERIZATION OF IMMUNE RESPONSES ELICITED DURING PKD INFECTION IN BROWN TROUT vs RAINBOW TROUT	
			ADP-P5	Chun-Hsin Wu	Computational Analysis of Immune Foods and Diets Based on Nutritional Similarity Measurement	
			ADP-P7	Haruka Tsukamoto	Characterization of culture supernatant of Streptococcus parauberis: hemolytic factor and leucocyte-stimulation in Japanese flounder	
			ADP-P8	J. W. Hodgkinson	Analysis of the immune interface of Mycobacterium marinum infections in the goldfish (Carassius auratus L.)	
			ADP-P9	M. Zhang	Depletion of conventional mature B cells and compromised specific antibody response in bovine Ig mu heavy chain transgenic mice	

Session Name and Date	Day	Time	ID No.	Presenter	Title	
Vertebrate innate/homeostatic response (VIH)	Thu	14:30	VIH-O1	S.MacKenzie	Behavioural fever is a synergic signal amplifying the innate immune response	
		14:50	VIH-O2	A. Frøyse	Inflammation - a co- factor in salmonid coronary disease?	
		15:40	VIH-O3	Whitney M. Gammill	Innate immune defenses in amphibian skin against the fungal pathogen Batrachochytrium dendrobatidis	
		16:00	VIH-O4	Dominika A. Przybylska	Beta-glucan enriched bath directly stimulates the wound healing process in common carp (Cyprinus carpio L.)	
		16:20	VIH-O5	T. Wulff	Immunological aspects of tissue regeneration in rainbow trout muscle - A proteomics and bioinformatics approach	
		16:40	VIH-O6	J.G.Schmidt	Innate immune responses to experimentally inflicted wounds in common carp (Cyprinus carpio L.) larvae and juveniles	
		17:00	VIH-O7	N.I. Vera-Jiménez	Carp head-kidney leukocytes display different oxygen radical production patterns after DAMP and PAMP stimulation.	
		17:20	VIH-O8	N.I. Vera-Jiménez	Modulation of fish fibroblast proliferation with B-glucan and hydrogen peroxide during scratch-wound healing in vitro.	
	Fri	10:30	VIH-O9	J Hikima	Expression of Type-I Interferon Gene Controlled by IRF3/7 Mediated through TBK1 in Japanese Flounder	★
		10:50	VIH-O10	A. Skjesol	Suppressors of Cytokine Signaling (SOCS) in Atlantic Salmon	
		Poster	VIH-P1	R. Belmonte	The Immune Response of Atlantic Salmon to Saprolegnia	
			VIH-P2	J. Wei	Cloning, characterization, and expression analysis of a thioredoxin from orange-spotted grouper (Epinephelus coioides)	
			VIH-P3	S. Tasumi	Candidate key molecule(s) determining host specificity of parasite on fugu, Takifugu rubripes	★
			VIH-P5	J. Kang	Skewed segregation of a microsatellite marker linked with homozyous lethality after metamorphose of olive flounder	
			VIH-P6	Seong Don Hwang	Molecular characterization, expression, and functional analysis of two thioredoxins in the black rockfish (Sebastes schlegelii)	
			VIH-P7	Sònia Rey Planellas	Emotional fever in the zebrafish (Danio rerio) during intra-peritoneal injection procedures.	
			VIH-P8	Josep V. Planas	Modulation of the transcriptomic effects of lipopolysaccharide in skeletal muscle by swimming in zebrafish (Danio rerio)	
			VIH-P9	A. Callol	European eel (Anguilla anguilla) 454 immunotrascriptome sequencing.	