O-021.

Kinetics of rainbow trout B cell response against VHSV: New insights from head kidney antibody repertoire studies

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Abstract
Upon infection, B-lymphocytes expressing antibodies specific for the intruding pathogen develop clonal responses triggered by pathogen recognition via the B-cell receptor. In teleost fish, in absence of lymph nodes the kinetics and anatomy of B-cell responses remain poorly characterized.
After B cells encounter their specific target, they differentiate into Ab secreting cells probably in spleen or kidney, and it is believed that mature plasma cells migrate and persist in the anterior kidney. Here, we undertook a comparative analysis of the variable heavy chain (VH) domain repertoires of the IgM and IgT in the head kidney of isogenic rainbow trout (*Oncorhynchus mykiss*) lines after primary infection, vaccination and boost with an attenuated strain of the rhabdovirus Viral Hemorrhagic Septicemia Virus (VHSV). We used a barcoded IgH cDNA sequencing approach to characterise the modifications of the antibody repertoire, through the analysis of VH usage in expressed V(D)J rearrangements, clonal diversity, frequency of clonotypes, clonotype distribution and sharing between individual fish and treatments, which is determined by convergent response to Ag and probability of generation by the recombination process. We found extensive modifications implicating most VH families one month after primary infection. In contrast, only
modest changes in terms of repertoire diversity and composition were observed 5 months postvaccination with the attenuated VHSV vaccine. A boost performed 5 months post vaccination induced additional alterations of the kidney IgH repertoire detected after one week, but they faded after one month. The IgM public response implicating VH5 and JH5, that we previously described in spleen, was again observed in all infected fish confirming its presence among B cells from the head kidney and its strong correlation with the response against the virus. Our results provide insights about the amplitude of the primary and secondary B cell response to VHSV infection in the tissue in which plasma cells and memory cells accumulate. Together with a standardisation effort of salmonid Ig genes annotation, these data pave the way for a better monitoring of B cell response kinetics in lymphoid tissues, an essential step to understand B memory mechanisms in fish.
Keywords: Antibody. B cells. Repertoire. Antiviral immunity. Memory

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O-022.

The differential regulating roles of mTOR signaling on IGM+ and IGT+ B cells in systematic and mucosal immune responses of rainbow trout

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Abstract
mTOR is an evolutionarily conserved serine/threonine kinase that plays a key role in cell growth and metabolism by sensing various environmental cues. In mammals, mTOR signaling functions as a metabolic checkpoint to influence the immune responses of T cells, B cells, dendritic cells, and other immune cells. However, the regulating role of mTOR signaling on fish cellular and humoral immunity is much less known. Our previous studies indicate that mTOR signaling also functions as the sensor and regulator of fish immunometabolism during pathogenic infection. To address the regulating roles of mTOR signaling on the immune responses of B cells in fish, we used rapamycin to inhibit the mTOR signaling of rainbow trout (*Oncorhynchus mykiss*) both *in vitro* and *in vivo*. After 3 days incubation *in vitro*, the relative percentages of IgM+ and IgT+ B cells within the whole isolated leukocytes were significantly decreased after rapamycin treatment. mTOR signaling of rainbow trout was significantly inhibited by rapamycin treatment, indicated by the inactivation of phospho-TOR and phospho-S6. mTOR signaling inhibition in rainbow trout *in vivo* resulted in poor growth performance and decreased feed efficiency, accompanied with which was the altered metabolism of glucose, glutamine and fatty acid. Compared to their counterparts in the control group, IgM concentration in the serum was significantly decreased, while IgT concentration in the gill mucus was significantly decreased after rapamycin treatment. Crucially, the relative percentages of IgM+ and IgT+ B cells in the head kidney fell by ~80% and ~20%, respectively, while the relative percentages of both IgM+ and IgT+ B cells in the spleen fell by ~50 % when mTOR signaling was inhibited. However, the relative percentages of IgM+ and IgT+ B cells in the gill fell by ~25% and ~75% after rapamycin treatment, respectively. Importantly, further studies indicated that, accompanied with the inhibition of mTOR signaling, both the proliferation of IgM+ B cells in the head kidney and the proliferation of IgT+ B cells in the gill were significantly inhibited. These data revealed the differential regulating roles of mTOR signaling on IgM+ and IgT+ B cells in fish systematic and mucosal immune responses for the first time, and further studies should be conducted to reveal the regulating mechanism of mTOR signaling on fish humoral immunity during pathogenic infection.
Keywords: mTOR signaling, B cells, rainbow trout, gill, head kidney.

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O-023.

Evidences for the immunomodulation by the melatonin hormone in pikeperch *Sander lucioperca*

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Abstract
The melatonin hormone is produced and secreted by the pineal gland during the dark phase of the photoperiod. It hence provides information such as time of the day and season for cells and organs. As in mammals, melatonin in fish is known to act on important physiological functions, including thermoregulation, reproduction and development. However, while well described in mammals, few studies have investigated its potential role on immune functions in teleost. In pikeperch, we defined in previous experiments a potential dual action of cortisol and melatonin hormones on immune defenses. In addition, we characterized daily cyclic activities of humoral innate immune markers that are correlated with the cyclic release of melatonin by the pineal gland.
Nocturnal peak of melatonin production and release is directly proportional to the length of the night and hence provides a direct transduction of night length. In order to deepen our knowledge on the immune

modulation by the melatonin hormone, we hypothesized that changing photoperiod influences the fish immune functions through the modulation of melatonin synthesis. The study thus investigated the effects of two natural photoperiod regimes simulating the fall and the spring in western Europe on melatonin secretion, stress and immune markers.
Daily cyclic activities were observed for plasma melatonin and cortisol, but also for several innate immune markers, including lysozyme, peroxidase and complement activities in plasma and phagocytic activity in spleen. Nocturnal plasma melatonin values were influenced by the seasonal simulated photoperiods with progressive increase or decrease for the photoperiods simulating the fall and the spring respectively. No photoperiod effect was detected on cortisol release. Moreover, the exposure to the fall-simulated photoperiod induced several effects on immune markers, including increases in lysozyme, peroxidase and complement activities. Analyses of immune-relevant gene expression are ongoing. Our results bring an additional evidence supporting the potential immunomodulatory action of the melatonin hormone in teleosts with a stimulation of the innate immunity following the increase in melatonin production in response to the fall-simulated photoperiod.
Keywords: Melatonin; photoperiod; immune system; circadian axis; pikeperch

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O-024.

Modifications of mucosal and systemic antibody repertoire after ERM nasal vaccination in rainbow trout

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Abstract
Bony fish have a dedicated mucosal immune system which comprises immunologically heterogeneous microenvironments armed with innate and adaptive immune components. In rainbow trout (*Oncorhynchus mykiss*), a nasopharynx-associated lymphoid tissue (NALT) was recently described as a diffuse network of myeloid and lymphoid cells located in the olfactory organ of fish. Teleost NALT presents IgM and IgT B cells in equal proportions and nasal mucus contains secreted IgM and IgT. Several studies have demonstrated that nasal vaccination is a very effective mucosal route to stimulate adaptive immune responses and high levels of protection against viral and bacterial pathogens in fish. However, the mechanisms underlying the observed protection are not well understood. We applied a barcoded 5′RACE IgH cDNA sequencing approach to investigate the structure of the systemic and mucosal rainbow trout immunoglobulin repertoire. Its analysis in control trout suggests different structures of IgM and IgT spleen and NALT repertoire, with restricted repertoire diversity in NALT. Nasal and intraperitoneal vaccination with enteric red mouth (ERM) vaccine also revealed unique dynamics of IgM and IgT repertoires at systemic and mucosal sites and the remarkable ability of nasal vaccines to induce spleen Ig responses. Our findings provide an important immunological basis for the effectiveness of nasal vaccination in fish and other vertebrate animals and will help the design of future nasal vaccination strategies.
Keywords: NALT, B cells, Immunoglobulin, Repertoire, Vaccine.

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O-025.

Under control: 20 IRAK3 variants regulate toll-like- and interleukin-1-receptor signalling in rainbow trout

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Abstract
The immune system of vertebrates enables the rapid and very efficient defence against microorganisms and viruses. Shortly after the detection of pathogens, amplifier mechanisms multiply various destructive activities, which may, however, also be directed against the host itself. An arsenal of inhibitory factors controls therefore the duration and extent of the immune response, restricts pathological events and restores homeostasis. For these reasons, it is essential that endogenous immune regulators are integrated in efficient health concepts in aquaculture.
However, immune inhibitors in teleost fish are still poorly explored, also due to the fact that the teleostean repertoire of immune inhibitors is more complex than the mammalian one. We found that the inhibitory kinase interleukin-1 receptor-associated kinase 3 (*irak3*) is present in more than 20 isoforms of varying length and nucleotide composition in rainbow trout. We elucidated the underlying genetic causes for this striking Irak3 diversity and profiled the expression of all *irak3* variants in healthy and infected rainbow trout. The obtained data revealed that the truncated *irak3* variants are expressed to a much greater extent than the full-length variants. The overexpression of selected full-length and truncated Irak3 variants in different cell models showed that the individual isoforms modulate the basal as well as the pathogen-induced activity of NF-kappaB with different efficiency. Confocal microscopy showed that the overexpression of the truncated Irak3 variant was associated with massive cell death, in contrast to the full-length variant. Based on these different observations, we assume that the multiple Irak3 variants do not represent sheer abundance. Rather, we hypothesize that different Irak3 variants could integrate specificly into the different cascades mediated by IL1R1 and more than a dozen TLRs. Certain Irak3 isoforms might suppress the inhibitory functions of their paralogs to steer the immune response from a suppressed to a reinducible state. Further analyses are on the way to test the hypotheses using suitable cell models and appropriate knock-out or knock-in techniques.
Keywords: Inhibitory factors; Innate Immunity; IRAK-3; Salmonids; Toll-like receptor signalling

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O-026.

Evidence of IgD-secreting plasmablasts and mucosa specific IgD molecular signatures in teleost gills and gut

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Abstract
IgD is an ancient immunoglobulin for which many aspects of its regulation and function remain unclear. Although usually expressed on the surface of