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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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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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