mature B cells along with IgM, a small subset of B cells undergo an unconventional IgM-to-IgD class switch and differentiate to IgD+IgMplasmablasts/plasma cells. In mammals, these cells are mostly found in nasopharyngeal lymphoid tissues and rarely in the intestine, although recent evidence suggests a role for IgD in gut immunity.
In teleost fish, a subset of IgD+IgM- B cells was previously reported in rainbow trout gills and in catfish blood. Here, we report that IgD+IgM- B plasmablasts also constitute a major B cell subset in the intestinal mucosa of rainbow trout. Thus, although IgD+IgM+ B cells represent almost 87% of the IgT- B cell subsets in the spleen, IgD+IgM- B cells accounted for 83% of IgT− B cells in the gut. A complete repertoire analysis of IgD in comparison to IgM and IgT performed in gills and gut as well as in spleen, confirmed the clonal expansion of IgD in these two mucosal sites but not in spleen. Remarkably IgD sequences in gills and gut share a common VDJ segment usage and had quite similar mutation profiles, different from those found in the spleen. Our data demonstrates that IgD-secreting plasmablasts represent a major B cell subset in rainbow trout gills and gut and that the IgD sequences derived from the clonal expansion of specific B cells found in these two tissues differ greatly from the IgD found in spleen that is mostly found on the cell surface in association with IgM.
Keywords: Rainbow trout, IgD, gills, gut, repertoire

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O-027.

Developmental immunotoxicology: What underlies the critical windows of exposure?

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Abstract
Endocrine disruptors in general and oestrogenic compounds in particular have been widely investigated in view of their effects on several physiological processes and, therefore, their ecotoxicologic relevance. The modulation of the immune system by oestrogens has increasingly sparked interest in the research community, that previously had been mainly centered on the reproductive effects of these hormones. In fact, since the industrialization an increasing variety of endocrine disruptors, such as oestrogenic endocrine disruptors, are retrieved in the environment. These oestrogenic endocrine disrupting chemicals (EEDCs) have also been suggested to increase the prevalence of autoimmune diseases and cancer. With regard to the high degree of similarities in the immune system of jawed vertebrates as well as the conserved immunomodulatory roles of oestrogen, environmental EEDCs possibly have the capacity to alter immune system functions of teleost fish, which may impair their capacity to fight infectious diseases and eventually may contribute, together with overfishing, to wild stock losses. Importantly, the most deleterious effects of EEDCs, both in mammals and teleosts, appear to arise when the exposure occurs during specific periods of the immune system ontogenesis, commonly referred as critical windows of exposure. However, in mammals and especially in teleost fish, these stages of the immune system development as well as the EEDC-action remain to be fully identified and characterised. The concept and the importance of developmental immunotoxicity is presented by addressing the mechanisms of oestrogenic regulation and the mode of action of EEDCs from an immunological perspective. Emphasis is given to the critical windows of development of the immune system during which EEDCs may alter the immune system development and function with long-term consequences on immunocompetence. Results from different classes of vertebrates are compiled, highlighting studies on teleost fish and their relevance for the human immune system. Additionally, new results on the effects of environmentally relevant concentration of exogenous estradiol exposure during European sea bass (*Dicentrarchus labrax*) development will be presented with regard to oestrogen's ability to trigger effects on immunocompetence, contributing to fill the gaps on vertebrate immunotoxicology.
Keywords: Developmental immunotoxicity; Immune system; Critical windows; Endocrine disruptors; Oestrogens

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O-028.

Contrasting reactivity of lymphocyte subsets to IL-15, IL-15L and IL-2 in rainbow trout

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Abstract
Interleukin (IL)-2 and IL-15 are used as immunostimulants in human medicine since they are known to induce activation, proliferation and maintenance of several lymphocyte subpopulations. In addition to IL-2 and IL-15, rainbow trout possess a third closely related functional cytokine IL-15like (IL-15L). Our previous studies have shown that the soluble form of the corresponding receptor (sIL-15Rα) enhances the secretion and/or stabilization of IL-15 and IL-15L *in vitro*. Therefore in this study, chimeric recombinant proteins of IL-15 and IL-15L with sIL-15Rα(IL-15-RLI and IL-15L-RLI, respectively)as well as IL-2 (as a free cytokine) were produced by using a baculovirus/insect cell system. These cytokines were then subjected to *in vitro* functional studies. For this, purified recombinant proteins were added to four different flow-sorted thymocyte sub-populations: CD4-Single Positive (SP), CD8-SP, CD4/CD8-Double Positive (DP) and Double Negative (DN) and to three lymphocyte sub-populations (CD4-SP, CD8-SP and DN) from intestine and spleen applying anti-CD8 and anti-CD4 monoclonal antibodies. Stimulated lymphocytes were then subjected to Western blot analysis to detect phosphorylation of STAT5, a molecule that can be found downstream the IL-2/15Rβγ chain signal transduction cascade, as a measure of lymphocyte activation. In addition, whole splenocytes were individually incubated with the recombinant cytokines and subjected to RT-qPCR analysis. A contrasting reactivity of lymphocytes to the three recombinant cytokines was observed in lymphocyte subsets from different lymphoid organs. While all of the tested lymphocyte subsets except DP thymocytes were activated by IL-15-RLI, DP thymocytes were only activated by IL-2 which stimulated all thymocyte subsets as well as the CD4-SP and CD8-SP splenocytes. A clear stimulatory effect of IL-15L-RLI was observed in DN thymocytes while DN and CD8-SP splenocytes were only activated at very high concentrations. RT-qPCR analysis confirmed the contrasting stimulatory effect of IL-15 and IL-15L chimeric proteins with IL-15Rα. In trout splenocytes, IL-15-RLI induced type 1 immune gene expression such as *IFNγ* and *Perforin*, but did not induce type 2 immune gene expression such as *IL-4/13*. In contrast, IL-15L-RLI induced type 2 immune gene expression but did not enhance type 1 immune gene expression. These results reveal the distinctive powers of the IL-2/15/15L family cytokines, and hold great promise for their practical use as well as for their capacity to help unravel the different arms of the fish immune system.
Keywords: Rainbow trout, IL-2 cytokine family, adaptive immunity, lymphocytes, recombinant proteins