Intestinal Mucosal Immunology of Salmonids

Response to Stress and Infection
and
Crosstalk with the Physical Barrier

Lars Niklasson
Institutionen för biologi och miljövetenskap
Naturvetenskapliga fakulteten
Till min familj
Dissertation abstract

Lars Niklasson (2013). Intestinal Mucosal Immunology of Salmonids – Response to Stress and Infection and Crosstalk with the Physical Barrier
Department of Biological and Environmental Sciences, Gothenburg University, Box 463, SE-405 30 Gothenburg

The effect of environmental factors and pathogens on the intestinal epithelium of fish has received increased attention in recent years. Studies focusing on effects of stress, nutrient uptake as well as vegetable ingredients in fish feed have all shown that the intestine is affected by environmental factors. The signs of inflammation during exposure to detrimental environmental conditions have brought to attention the local immune system in the gut. The gut is further one of the main routes for pathogen infection in fish. Therefore this thesis aims at investigating the mucosal immune factors and systems that are affected by environmental stressors and pathogen interactions.

In this thesis the effect of long term environmental stress on the mucosal intestinal epithelium was investigated. Results showed an ongoing inflammation in the intestine that was manifested as a compromised barrier integrity, infiltration of immune cells and an affected immune response. Atlantic salmon was co-habitant infected with infectious pancreatic necrosis virus as well as immune challenged with the viral mimicker, double stranded RNA Poly I:C, where after the mucosal immune response was studied. Both treatments clearly demonstrated an antiviral response including alterations of IFN type I and the Mx protein. When the fish were exposed to a stressor and immune stimulation in combination, the fish immune response was delayed. This stresses the importance of minimize stressful situations for the animals in, for example aquaculture. The demonstrated increase in intestinal epithelial permeability together with inductions of the mucosal immune system raises the question of whether stress or inflammation is the causative agent of the barrier dysfunction.

To address this, the effect of the immune system on the intestinal epithelium was assessed using an in vitro Ussing chamber approach in which the intestinal epithelia was exposed to recombinant cytokines. Exposure to IL-1β and IL-6 showed negative impact on the intestinal permeability, suggesting that the immune system of the fish is contributing to the inflammation seen during prolonged stress. Further, the tight junction proteins create an extracellular network between the epithelial cells and by that controls the intestinal paracellular permeability was shown to be affected by the two cytokines. The interactions between stress, the immune system and the epithelial barrier function are therefore highly complex and important for our understanding of the physiology of health, welfare and disease.

Keywords: Inflammation, IPNV, Poly I:C, Recombinant cytokines, CD8, MHC-I, Claudins, Permeability, Environmental stress, Cortisol, DNA constructs, IL, IFN
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>MALT</td>
<td>Mucosal-associated lymphoid tissue</td>
</tr>
<tr>
<td>GALT</td>
<td>Gut-associated lymphoid tissue</td>
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<tr>
<td>M cell</td>
<td>Microfold cell in the Payers patches</td>
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<tr>
<td>NF-κB</td>
<td>DNA transcription factor Nuclear Factor κB</td>
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<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>IFN</td>
<td>Interferon</td>
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<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
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<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
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<tr>
<td>Mx</td>
<td>Myxovirus resistance (by origin)</td>
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<tr>
<td>CD</td>
<td>Clusters of differentiation antigen on T cells. Different types on different cell types</td>
</tr>
<tr>
<td>B cell</td>
<td>Antibody producing lymphocyte</td>
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<tr>
<td>T cell</td>
<td>Lymphocyte derived from the thymus (T)</td>
</tr>
<tr>
<td>T&lt;sub&gt;h&lt;/sub&gt;</td>
<td>T helper cells</td>
</tr>
<tr>
<td>T&lt;sub&gt;c&lt;/sub&gt;</td>
<td>Cytotoxic T cells</td>
</tr>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD8 positive – associated with Tc</td>
</tr>
<tr>
<td>CD4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD4 positive – associated with Th</td>
</tr>
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List of papers which are referred to in the text by their Roman numbers

I
Disturbance of the intestinal mucosal immune system of farmed Atlantic salmon (Salmo salar), in response to long-term hypoxic conditions. Fish and Shellfish Immunology (2011). Niklasson L.; Sundh H.; Fridell F.; Taranger GL.; Sundell K. Fish and Shellfish Immunology 31:1050-4648

II
High stocking density and poor water quality disturbs the intestinal physical and immunological barriers of the Atlantic salmon. Sundh H.; Niklasson L.; Finne-Fridell F.; Ellis T.; Taranger G L., Pettersen E F.; Wergeland H I.; Sundell K. (Under revision for publication in Fish and Shellfish Immunology)

III
Modulation of innate immune responses in Atlantic salmon by chronic hypoxia-induced stress (2013). Bjørn Olav Kvae; Koestan Gadan; Frode Finne-Fridell; Lars Niklasson; Henrik Sundh; Kristina Sundell; Geir Lasse Taranger; Oystein Evensen. Fish and Shellfish Immunology 34:1095-9947

IV
Cortisol effects on the intestinal mucosal immune responses during cohabitant challenge with IPNV in Atlantic salmon (Salmo Salar). Niklasson L.; Sundh H.; Olsen R-E.; Jutfelt F.; Skjødt K.; Nilsen T O.; Sundell K. (Submitted for publication in PLOS ONE)

V
Recombinant cytokines interleukin 1 beta and interleukin 6 increases intestinal epithelial permeability in Rainbow trout (Oncorhynchus mykiss). Niklasson L.; Sundell K.; Martin S.; Secombes C.; Sundh H. (Manuscript)
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### ACKNOWLEDGEMENTS

### REFERENCES