Interactions between parasites and their fish host

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Outbreaks of parasites not only threaten intensive aquaculture systems such as salmon farming, but also semi-intensive carp systems in natural ponds in Central Europe. Although chemotherapeutants can be effective, immunostimulation and/or genetic selection for innate disease resistance certainly are more sustainable approaches. Research into these approaches, however, needs well-defined parasite infection models, allowing for reliable challenge experiments and monitoring of the efficacy of treatments. In addition, parasite infection models also serve as models for a better understanding of immunological concepts. After all, whole animal infection experiments remain essential for a true understanding of the immune system of fish. The workshop summarized here, comprised presentations on host-parasite studies in common carp (*Cyprinus carpio* L.), as part of a European Community (EC)-funded research training network, titled 'Integrated approach to the innate immune response to parasites in fish' (PARITY).

Taken from a live gene bank were six carp lines with a genetic difference in natural survival. When infected with the blood parasite *Trypanoplasma borreli*, genetic differences in disease resistance were detected. Subsequent studies have been aimed at explaining the genetic lay-out of observed differences and their possible correlation with the carp innate immunity:

Natural antibody levels, considered effective against many different pathogens because of its polyreactivity, were present in different serum levels in the different carp lines, suggestive of a genetic control. These genetic differences were independent of antigen, age and environment.

Alpha-2-macroglobulin (a2M), a non-specific protease inhibitor, was studied. Four genetically different forms of a2M were found at the cDNA level, directing future studies towards finding a putative correlation with genetic disease resistance. Serum anti-protease activity decreased during the late phase of infection, while a2M expression increased.

C-reactive protein (CRP), considered part of the acute phase response (APR), was studied by ELISA making use of specific antibodies

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raised against purified carp CRP. However, the role of CRP in the APR of carp remains to be established.

All these innate immune factors were studied to find an early warning system for resistance against fish parasites. To further detail the innate immune response, primers were designed for real-time quantitative (RQ)-PCR analysis of chemokines and pro- and anti-inflammatory cytokines, initially for the immune response to *Trypanoplasma borreli*. Making use of these RQ-PCR primers, a predictive model of gene expression induced only by mechanical injury of fish skin was studied. This was done to unravel the confounding effects of injury and ectoparasite-specific molecules on the immune system. Indeed, early expression of several chemokines and cytokines was up-regulated simply by injury to the skin. With this information at hand, follow-up studies now focus on the expression of immuno-regulatory genes in response to ectoparasite-specific molecules from *Ichthyophthirius multifiliis* and *Argulus japonicus*.

Immunohistochemistry and a novel cell migration assay indicated that primarily neutrophilic granulocytes migrate towards the places of epithelial injury. For a full understanding of the innate immune response against parasites on the skin and in the intestinal tract, studies on the mucosal layer are essential. Mucus glycoprotein (mucin) genes were identified for expression studies in response to intestinal parasites such as *Goussia carpelli* (coccidia) and *Bothriocephalus acheilognathi* (tapeworm). Maybe an early warning system based on the mucosal immune response can be developed, which would certainly prove useful for non-invasive diagnosis of intestinal infections in fish.

Parasites can survive for long periods in/on the fish, probably maintaining themselves by immunomodulation of the host responses. Low level infections often do not have major direct effects but indirectly do cause stress-induced changes, such as programmed cell death (apoptosis) of leukocytes. Genes were identified for RQ-PCR studies on (stress-induced) apoptosis of carp leukocytes, allowing for detailed studies on parasite immunomodulation.

At present we are experiencing a rapid increase in our knowledge of genes involved in the fish host' immune response against parasites. Future studies should also aim at studying the expression of genes from parasites, which would take us a step further into the proteomics era, but *in vivo* parasite infection studies will remain essential to a true understanding of host-parasite interactions. The fish parasite infection models described here represent, without exception, natural infections in fish. The outcomes therefore, can be interpreted and applied in a direct manner. We hope that this workshop contributed to the understanding of fish host-parasite interactions and will stimulate further research in this direction.