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General and family-specific gene expression responses to viral hemorrhagic septicaemia virus infection in rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

The ability of rainbow trout (*Oncorhynchus mykiss*) to respond successfully to infection by viral hemorrhagic septicaemia virus (VHSV) is expected to involve a large number of biochemical processes. We hypothesized that this would be reflected at the gene expression level in infected fish, and we tested it by examining gene expression levels in the head kidney of trout at a genome-wide scale with a 16K cDNA microarray for salmonids. Expression levels were recorded during 16 days following bath challenge. The challenge experiment included a relatively low susceptibility (32% survival following challenge) and a relatively high susceptibility (18% survival following challenge) trout family that were both split into a group exposed to virus and a non-exposed control group. In total, 939 genes were differentially expressed between infected and non-infected fish (FDR $p = 0.05$). Five groups of Gene Ontology categories were involved in immune-related processes and over-represented in infected fish: (i) stress and defense response, (ii) NFkappaB signal transduction, (iii) response to non-self, (iv) antigen processing and presentation, and (v) proteasome complexes. The first four categories were also over-represented among the 642 differentially expressed genes in the low-susceptibility trout family but not among the 556 differentially expressed genes in the high-susceptibility trout family. Expression profiles for most immune genes discussed showed increased transcription from day 3 post-challenge. The results suggest that the innate immune system may play an important role in the successful response to VHSV in rainbow trout. In addition, the results indicate that a superior regulation of the transcription of several key innate immune-related genes contribute to the increased survival in resistant fish.

1. Introduction

Disease resistance is a complex trait. Therefore, the ability of the rainbow trout (*Oncorhynchus mykiss*) to respond successfully to infection by viral hemorrhagic septicaemia virus (VHSV) is expected to involve a large number of biochemical processes. The complex interactions between virus and host can be studied by investigating changes in gene expression of the host following viral infection (Katze et al., 2008). Understanding these interactions is necessary to identify processes that have a positive effect on trout disease resistance (or a negative effect on the virulence of VHSV) on a longer term.

It has already been established that expression levels of specific immunogenes change in rainbow trout following infection by or vaccination against rhabdoviruses (e.g., Purcell et al., 2010; Cuesta and Tafalla, 2009; Tafalla et al., 2005). Responses by the trout included increased transcription of genes coding for interferons (IFNs) (Purcell et al., 2010), interleukin 1β (IL-1β), major histocompatibility complex I (MHC Iα) (Cuesta and Tafalla, 2009), interleukin 8 (IL-8) and transforming growth factor β (TGF-β) (Tafalla et al., 2005). These results provide important information for specific genes that are known to play a role in responses to viral infection. We decided to study infection responses on a genome-wide scale, since a large part of the genome is active in several biochemical processes, including the immune defense (Hyatt et al., 2006). Thus, by analyzing a large number of genes simultaneously, we may gain

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considerable additional knowledge on biochemical processes that are activated by VHSV in trout. It is possible to run such an analysis on a whole-genome scale using the 16,000 spot salmonid cDNA microarray (von Schalburg et al., 2005).

Our first hypothesis was that VHSV infection induces a response in the trout host at the gene expression level. We therefore expected to see differential expression between infected fish and non-infected control fish in tissue with immune function (head kidney) for some of the transcripts on the 16K salmonid cDNA microarray. Gene expression levels have been shown to vary according to genotype in several species (e.g., Schadt et al., 2003; Brem et al., 2002). In addition, survival following VHSV infection varies between families of rainbow trout, demonstrating genetic variation for resistance to VHS (Henryon et al., 2002, 2006). Therefore, our second hypothesis was that infection responses at the gene expression level differed between trout families of high and low mortality following challenge with VHSV.

To test the two hypotheses, we exposed trout with different susceptibility to VHS and measured their gene expression levels for approximately 16,000 transcripts during the first 16 days following infection. We infected a family of low susceptibility (LS) and a family of high susceptibility (HS) rainbow trout with VHSV, and the results supported our hypotheses and improved our understanding of what constitutes a successful response to VHSV infection in rainbow trout. The individual fish from which we obtained gene expression profiles were removed from the experiment, and consequently their fate in response to the virus challenge is necessarily undetermined. However, the performance of their siblings provides us with a statistical prediction of their expected fate, which we use to define the LS family and the HS family. We observed both general infection responses and family-specific infection responses in the trout. The immune responses can generally be considered at three levels: (1) inter-cellular, (2) intra-cellular and (3) gene transcription. Overall, our results highlight the importance of early (innate) immune response mechanisms in trout for the outcome of an infection. This outcome is determined by the concerted action of a larger number of factors.

2. Materials and methods

2.1. Experimental design

We assessed the gene expression profiles of an LS and an HS full-sib family of rainbow trout during 16 days following challenge with viral hemorrhagic septicaemia virus. We set up four treatment groups: each of the two trout families was divided into a group that was challenged with VHSV and a non-challenged control group. Each of the four treatment groups contained eight aquaria with ca. 30 fish each. Six of these aquaria were used for sampling, and two aquaria were used exclusively to record mortality. Six fish from each treatment group were sampled at time 0 and 6 h and 1, 2, 3, 5, 7, 10 and 16 days following challenge. Of the six fish sampled at each time point, all were used for virology analyses while five were chosen at random for gene expression analyses. As fish died from VHS in the challenge aquaria, we removed fish from the corresponding control aquaria to keep the densities equal. With this design we could determine whether there were general and family-specific infection effects that would deserve closer attention.

2.2. Trout families

A low-susceptibility and a high-susceptibility rainbow trout family were identified in a pilot study. In the pilot challenge, we included ten full-sib rainbow trout families from a commercial Danish breeding stock, and they were challenged with VHSV at an average weight of 5 g. Based on the survival results in the pilot experiment, two extreme families were picked out for the final challenge. The family with the lowest mean survival weighted based on the initial number of fish (5%) represented trout of relatively high susceptibility, and the family with the highest weighted mean survival (55%) represented trout of relatively low susceptibility. During the final challenge experiment on which this study is based, the trout weighted 8.5 g on average in the resistant family and 10.5 g in the susceptible family. The weighted mean survival after 28 days for all eight aquaria was 18% in the HS family and 32% in the LS family (Fig. 1). During experiments, the fish were kept in running tap water in 8 L aquaria at 9–10 °C. They were fed low rations of feed daily (BioMar, 1.5% bodyweight).

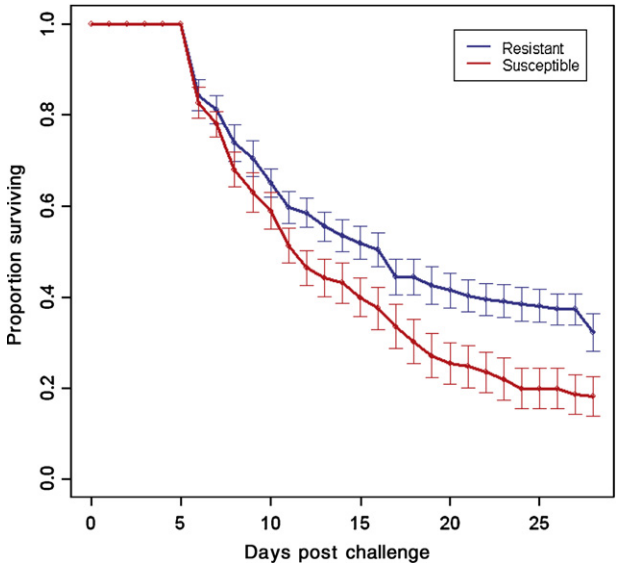

Fig. 1. Proportion of fish surviving VHSV bath challenge. The lines represent mean values weighted for all eight aquaria within each family. Error bars show standard errors for individual time points. At the termination of the experiment, the weighted mean survival for the family referred to as 'low-susceptibility' was 32% and for the family referred to as 'high-susceptibility', it was 18%.

2.3. Challenge with VHSV

The trout were exposed to VHSV using a bath challenge as described in Lorenzen et al. (2000). Each challenge aquarium contained 8 L of water and received 1 mL of highly virulent, low passage VHSV (cell culture supernatant, VHSVB 3592B 2nd passage BF-2 (bluegill fry) cells). The titer of the cell culture supernatant was 6.5×10^9 $TCID_{50}$/mL. The supernatant was diluted 1:50 before inoculation of 1 mL into the aquaria corresponding to a final concentration of 1.6×10^4 $TCID_{50}$/mL of aquarium water. Control aquaria received 1 mL cell culture medium (MEM). The water flow was turned off during bath challenge. After 2 h, the water flow was re-established.

2.4. Sampling

Six fish were sampled at each time point following challenge, one fish from each of the six sampling aquaria in a treatment group. The fish were anaesthetized in 0.01% benzocaine (ethyl *p*-aminobenzoate) before they were sacrificed by cutting the vertebrate column. The head kidney was immediately removed and stored in RNAlater (Qiagen). We sampled fish that were not visibly moribund, since we assume that the immune system and overall physiology of those fish have deteriorated beyond normal functionality. In addition, dead fish were recorded and sampled daily over