Developmental and Comparative Immunology (2008) 32, 1539–1560

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Resistance to amoebic gill disease (AGD) is characterised by the transcriptional dysregulation of immune and cell cycle pathways

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Received 21 March 2008 Received in revised form 3 May 2008; accepted 21 May 2008
Available online 20 June 2008

KEYWORDS
Microarray;
Disease resistance;
Salmonid;
Parasite infection;
Acquired immunity

Summary
Amoebic gill disease (AGD) is a parasite-mediated proliferative gill disease capable of affecting a range of teleost hosts. While a moderate heritability for AGD resistance in Atlantic salmon has been reported previously, the mechanisms by which individuals resist the proliferative effects remain poorly understood. To gain more knowledge of this commercially important trait, we compared gill transcriptomes of two groups of Atlantic salmon, one designated putatively resistant, and one designated putatively susceptible to AGD. Utilising a 17k Atlantic salmon cDNA microarray we identified 196 transcripts that were differentially expressed between the two groups. Expression of 11 transcripts were further examined with real-time quantitative RT-PCR (qPCR) in the AGD-resistant and AGD-susceptible animals, as well as non-infected naïve fish. Gene expression determined by qPCR was in strong agreement with the microarray analysis. A large number of differentially expressed genes were involved in immune and cell cycle responses. Resistant individuals displayed significantly higher expression of genes involved in adaptive immunity and negative regulation of the cell cycle. In contrast, AGD-susceptible individuals showed higher expression of acute phase proteins and positive regulators of the cell cycle.

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0145-305X/$ - see front matter
doi:10.1016/j.dci.2008.05.013

Combined with the gill histopathology, our results suggest AGD resistance is acquired rather than innately present, and that this resistance is for the most part associated with the dysregulation of immune and cell cycle pathways.

Introduction

Many pathological conditions in vertebrates result in cell proliferation and changes in cellular architecture. Sometimes such conditions are initiated by a pathogen, as is the case with the parasite-mediated proliferative condition: amoebic gill disease (AGD). Proposed to be caused by the protozoan *Neoparamoeba perurans* [1,2], AGD affects cultured teleost species including Atlantic salmon (*Salmo salar*) [2,3], rainbow trout (*Oncorhynchus mykiss*) [4]; turbot (*Scophthalmus maximus*) [5]; coho salmon (*Oncorhynchus kisutch*) [6]; and seabass (*Dicentrarchus labrax*) [7]. Following initial infection, AGD causes extensive alterations in gill morphology: severe epithelial hyperplasia, hypertrophy, oedema and interlamellar vesicle formation [3]. At present, the only successful treatment for fish affected by AGD is freshwater bathing [8]. However, as fish are continuously being re-infected, bathing needs to be repeated up to 12 times in a production cycle. Due to the high financial and logistic costs associated with this practice, bathing is not considered a viable long-term management solution.

Previous studies have suggested moderate heritability for AGD resistance within Atlantic salmon [9]. Enhancing this genetic resistance through selective breeding has become a major research focus. While improvements are possible using quantitative phenotype-based selection, the use of marker-assisted selection (MAS) is preferable. MAS is most appropriate for traits in which the phenotype is difficult or expensive to measure, such as disease resistance. To facilitate a MAS programme, genes (or their correlated markers) associated with resistance need to be identified. Such an undertaking is complicated by the fact that disease resistance—as a trait—is often complex and usually under polygenic control. For example, resistance to the parasite *Gyrodactylus salaris* in Atlantic salmon is associated with multiple-genomic regions, presumably spread over a number of genes [10]. A similar situation is observed for the loci controlling resistance to *Ceratomyxa shasta* in rainbow trout [11].

Mechanisms controlling AGD resistance in Atlantic salmon remain poorly understood. Studies have demonstrated that some Atlantic salmon previously infected with AGD will develop resistance upon subsequent re-infection and that this resistance is associated with the presence of anti-*Neoparamoeba* spp. antibodies [12]. Furthermore, a significant association between AGD resistance and allelic variation within the major histocompatibility (MH) class II alpha (*Sasa-DAA*) chain has also been described [13]. Despite these encouraging associations, the main molecular mechanisms controlling AGD resistance are yet to be identified.

Host response to AGD has been studied extensively. At the transcriptome level, Atlantic salmon infected with AGD show up-regulation of the pro-inflammatory cytokine interleukin-1β (IL-1β) within the gill tissue [14,15], and the transcription factor CCAAT/enhancer binding protein β (C/EBPβ) within the anterior kidney [16]. While suggestive of an acute phase response (APR), previous studies have reported either no, or only modest inductions of acute phase proteins following AGD infection [14,16–19]. Furthermore, upon first infection by AGD Atlantic salmon demonstrate a localised host immunosuppression [16], including down-regulation of genes involved in the MH class I and class II pathways [19]. More recently, it has become apparent that genes involved in apoptosis and cellular proliferation pathways may have an important role in the host response to AGD, at least upon first infection [16,17].

DNA microarrays are an important tool for investigating transcriptional changes within many aquatic organisms. In salmonids, microarrays have been used to examine responses to stress [20], bacterial infection [21,22], maturation [23], vaccination [24] and cytokine stimulation [25,26]. Microarrays have also become popular for identifying genes associated with disease resistance [27,28]. The present study has used a recently developed Atlantic salmon cDNA microarray [26] to compare the transcriptome response of AGD-resistant and AGD-susceptible Atlantic salmon following natural infection.

Materials and methods

Field AGD challenge

On 17 August 2006 a total of 2375 mixed-sex Atlantic salmon smolts (212.5 ± 52.3 g) were stocked into a single ($10 \times 10\,m^2$) sea-cage located within a commercial Atlantic salmon farm in Southern Tasmania. Fifteen hundred of these were previously passive integrated transponder (PIT) tagged and represented 140 half-sib families. The population was allowed to become naturally infected with AGD until 26 September 2006. The entire population was then scored for severity of AGD using the standard industry scoring method, which estimates the number of visible gross lesions on the gill surface [29] and assigns a score of between 0 and 5 to each individual, where 0 represents no visible lesions and 5 represents heavily infected gills. Following scoring, all fish were bathed in freshwater using standard industry protocols. After bathing, fish were again allowed to become naturally infected with AGD to a severe commercial score (10% of the cage population scoring over gill score 5) until 6 December 2006 when the gill scoring and bathing process was repeated. Following the second bathing, the remaining 1822 fish were allowed to become re-infected for the third time and their severity of AGD infection scored after 50 days. The fish, however, were not bathed and were allowed to become more severely infected with AGD and eventually succumb to the disease. The trial was then terminated on 16