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The expression of immune-regulatory genes in rainbow trout, *Oncorhynchus mykiss*, during amoebic gill disease (AGD)

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Abstract

Amoebic gill disease (AGD) is an ectoparasitic disease caused by infection with the protozoan *Neoparamoeba* sp. and is characterised by epithelial hyperplasia that manifests as gill lesions. In order to examine the nature of the immune response to AGD, the expression of a range of immune-regulatory genes was examined in naïve uninfected rainbow trout, *Oncorhynchus mykiss*, and naïve rainbow trout subjected to a laboratory-induced AGD infection. The immune-regulatory genes examined were interleukin-1 beta isoform 1 (IL-1β1), tumour necrosis factor alpha isoforms 1 and 2 (TNF-α1, TNF-α2), interleukin-8 (IL-8), transforming growth factor beta isoform 1 (TGF-β1), inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), major histocompatibility complex beta chain (MHC-II β-chain) and T-cell receptor beta chain (TCR β-chain). Immune-regulatory genes that were up/down-regulated in AGD-infected trout compared to uninfected controls at 0, 7, and 14 days post-inoculation (p.i.) in gill, liver and anterior kidney tissue were initially identified by means of semi-quantitative RT–PCR. Up/down-regulated immune-regulatory genes were subsequently quantitated and validated by real-time RT–PCR (qRT–PCR). The extent of AGD-associated pathology was consistent amongst all AGD-infected trout at 7 days p.i. and increased considerably by 14 days p.i. At both 7 and 14 days p.i. IL-1β1 and iNOS gene expression was significantly up-regulated in the gills, and IL-8 was significantly up-regulated in the liver of AGD-infected trout at 7 days p.i. These data demonstrate the involvement of the immune response to AGD at the molecular level, and indicate the importance of this response at the site of infection and the possible involvement of a systemic immune response.

Keywords: Immune-regulatory gene expression; Cytokine; Amoebic gill disease; AGD; Rainbow trout

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1. Introduction

Amoebic gill disease (AGD) is an ectoparasitic infection of fish gills due to infestation of the gills with the protozoan *Neoparamoeba* sp. Clinical signs of AGD include lethargy, flared opercula, obvious respiratory distress, loss of equilibrium, and mortality if left untreated [1]. Strongly associated with these clinical signs is the presence of grossly visible pale multifocal gill lesions that, when examined histologically, are characterised by epithelial hyperplasia, lamellar fusion and the formation of interlamellar crypts [2,3].

Neoparamoeba spp. have been isolated from several cultured marine fish species during AGD outbreaks throughout many different geographical locations worldwide [4]. However, AGD and the ensuing cost of the current treatment, freshwater bathing, have negatively impacted on the production of Atlantic salmon, *Salmo salar*, in Tasmania, Australia, more so than anywhere else in the world.

Little is known about the involvement of the immune response in AGD infection. Previous investigations have reported the detection of serum anti-*Neoparamoeba* sp. antibodies in farmed Atlantic salmon [5]. However, Atlantic salmon infected with *Neoparamoeba* sp. do not necessarily develop serum anti-*Neoparamoeba* sp. antibodies [5], and previously infected fish are not protected from reinfection upon subsequent re-exposure to *Neoparamoeba* sp. [6]. Histological changes associated with infection of the gills with *Neoparamoeba* sp. during AGD are relatively well characterised [3,7–10]. Besides the obvious epithelial hyperplasia that presents as grossly visible pale multifocal gill lesions, there is the less apparent infiltration of leucocytes and oedema associated with a local inflammatory response. This migration of leucocytes is highly dependent on the stage of infection and age of the lesion [3,7,8,10,11]. Lesion-associated leucocytes are predominantly found in the central venous sinus (CVS) where they supposedly extravasate into the lesions and are often found in close association with amoebae in interlamellar crypts [10,11]. These leucocytes appear to participate in lesion repair [8–11]. However, their role in other immune responses in AGD is unknown. Although knowledge of the host immune response in AGD is limited, it is well known that various cytokines and other immune-related factors co-ordinate the immune response to various pathogens.

The identification and characterisation of numerous fish cytokine and immune-regulatory genes in recent years has allowed the study of these genes during disease processes. Thus, the number of studies on the expression of immune-regulatory genes in fish infected with various bacterial, viral and parasitic pathogens, and their involvement in specific disease processes, is rapidly increasing [12–21]. Of particular relevance to the present study are investigations into the expression of rainbow trout, *Oncorhynchus mykiss*, immune-regulatory genes during infection with the ectoparasites *Ichthyophthirius multifiliis* [18,19] and *Gyrodactylus derjavini* [20,21]. Infection with the parasitic ciliate *I. multifiliis* resulted in an increased expression of interleukin (IL)-1β, tumour necrosis factor (TNF) and IL-8 at the site of infection, the skin. IL-1β and IL-8 were also up-regulated in the anterior kidney and spleen, but to a lesser extent [19]. However, the greatest increase in expression was observed in the skin at 4 days post-infection (p.i.), where IL-1β expression was up-regulated relative to controls by 17.8 fold [19]. The immunological importance of the site of infection was also highlighted by Sigh et al. [18], who showed that genes encoding complement factor C3, major histocompatibility complex (MHC)-II, immunoglobulin (IgM) and inducible nitric oxide synthase (iNOS) were up-regulated in the skin of trout, *O. mykiss*, infected with *I. multifiliis*. Similarly, IL-1β isoforms 1 and 2, the type II IL-1 receptor (IL-RII, 'decoy receptor'), TNF-α1 and iNOS gene expression were shown to change in skin tissue during parasitic infection of rainbow trout with the monogenean *G. derjavini* [21].

In the present study we examined the expression of selected rainbow trout cytokine and immune-regulatory genes in gill, liver and anterior kidney tissue during infection with the parasitic amoeba *Neoparamoeba* sp. to gain further knowledge of the involvement of AGD-related local and systemic immune responses in this salmonid species.