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Granulomatous inflammation in ginbuna crucian carp *Carassius auratus langsdorfii* against *Mycobacterium gordonae*

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ABSTRACT

In this study, we investigated the immune responses against *Mycobacterium gordonae* in ginbuna crucian carp. Cumulative mortality of ginbuna injected with 2.0×10^7 CFU of *M. gordonae* was 50% at 170 days post-infection. CD4-1, CD8α, T-bet and IFNγ2 gene expression levels were significantly upregulated in ginbuna injected with 1.9×10^8 CFU of *M. gordonae* at 21 and 28 days post-infection. The CD4-2 level did not change during the experiment. Granulomatous responses consisted of central macrophage accumulation and surrounding lymphocytes, and Ziehl-Neelsen-positive bacteria were observed in the trunk kidney of the challenged fish. Immunohistochemistry using anti-ginbuna IFNγs and anti-ginbuna CD4-1 polyclonal antibody revealed that the marginal lymphocytes were positive for CD4-1, and the IFNγ-producing cells surrounded the mycobacterial cell-laden phagocytes. These results suggest that CD4-1$^+$ cells and IFNγ2 play important roles in the granulomatous inflammation against Mycobacterial infections in teleosts.

1. Introduction

Mycobacteriosis causes severe economic losses to fish production in the aquacultural industry worldwide. To date, more than ten mycobacterial species have been reported to affect cultured, ornamental, and wild fish (Gauthier and Rhodes, 2009). *Mycobacterium gordonae* has been isolated from apparently healthy ornamental fish, such as goldfish (*Carassius auratus auratus*), guppies (*Poecilia reticulata*), freshwater angelfish (*Pterophyllum scalare*), and Cockatoo cichlids (*Apistogramma cacatuoides*), in aquaria (Pate et al., 2005; Novotny et al., 2010). Heavy mortalities caused by *M. gordonae* infections have also been observed on many fish farms for giant gourami (*Osphronemus gourami*) and guppies in southeast Asian countries (Dana et al., 1996; Areechon et al., 2001; Sakai et al., 2005). The affected guppies showed inappetence, sluggish swimming, fin erosion, and skin ulceration, with granulomas often observed in their livers, intestinal connective tissue, and dermal and subdermal tissues (Areechon et al., 2001). Similar granulomas have also been found in other fish species infected with *M. gordonae* (Novotny et al., 2010).

Mycobacteria are Gram-positive, aerobic, acid-fast, and non-motile bacteria that are capable of intracellular parasitism. Pathogenic mycobacteria can survive within the hostile environment of the macrophages, and these mechanisms are well-studied in *M. tuberculosis* (Pieters, 2008). Mycobacteria can avoid initial phagosomal degradation by producing catalases, peroxidases, and superoxide dismutases that eliminate oxygen radicals (Bartos et al., 2004). In addition, they can survive macrophages by secreting protein kinase G out of the phagosome to inhibit phagosome-lysosome fusion (Nguyen and Pieters, 2005). The infected macrophages are surrounded by CD4$^+$ T cells, CD8$^+$ T cells, B cells, macrophages, neutrophils, and fibroblasts, resulting in a granuloma, the typical structure of mycobacteriosis (Co et al., 2004). In mammals, CD4$^+$ T cells play critical roles in initiating, constructing, and maintaining the granuloma. Mice that are genetically deficient in CD4$^+$ T cells form aberrant lesions that cannot control the mycobacteria (Saunders et al., 2002), and the depletion of CD4$^+$ T cells during a persistent infection reactivates the mycobacteriosis (Scanga et al., 2000). Interferon-γ (IFNγ), produced by CD4$^+$ T cells, is an important cytokine for forming granulomas and killing bacteria. Complete deficiency of the IFNγ receptor is associated with poor granuloma formation (Ottenhoff et al., 2005). IFNγ upregulates more than 200 genes to produce antimicrobial molecules, such as oxygen free radicals and nitric oxide, resulting in mycobacterial death in the intracellular environment (Cavalcanti et al., 2012). Thus, it had been considered that granulomas were essential to preventing mycobacterial dissemination

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