ELSEVIER

Transcriptome profiling of adult zebrafish at the late stage of chronic tuberculosis due to *Mycobacterium marinum* infection[☆]

Annemarie H. Meijer[a,*], Fons J. Verbeek[b], Enrique Salas-Vidal[a],
Maximiliano Corredor-Adámez[a], Jeroen Bussman[a,b], Astrid M. van der Sar[c],
Georg W. Otto[d], Robert Geisler[d], Herman P. Spaink[a]

[a] *Institute of Biology, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands*
[b] *Leiden Institute of Advanced Computer Science, Leiden University, Niels Bohrweg 1, 2333 CA Leiden, The Netherlands*
[c] *Department of Medical Microbiology, VU Medical Centre, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands*
[d] *Max-Planck Institut für Entwicklungsbiologie, Spemannstrasse 35–37, 72076 Tübingen, Germany*

Abstract

The *Mycobacterium marinum*–zebrafish infection model was used in this study for analysis of a host transcriptome response to mycobacterium infection at the organismal level. RNA isolated from adult zebrafish that showed typical signs of fish tuberculosis due to a chronic progressive infection with *M. marinum* was compared with RNA from healthy fish in microarray analyses. Spotted oligonucleotide sets (designed by Sigma-Compugen and MWG) and Affymetrix GeneChips were used, in total comprising 45,465 zebrafish transcript annotations. Based on a detailed comparative analysis and quantitative reverse transcriptase-PCR analysis, we present a validated reference set of 159 genes whose regulation is strongly affected by mycobacterial infection in the three types of microarrays analyzed. Furthermore, we analyzed the separate datasets of the microarrays with special emphasis on the expression profiles of immune-related genes. Upregulated genes include many known components of the inflammatory response and several genes that have previously been implicated in the response to mycobacterial infections in cell cultures of other organisms. Different marker genes of the myeloid lineage that have been characterized in zebrafish also showed increased expression. Furthermore, the zebrafish homologs of many signal transduction genes with relationship to the immune response were induced by *M. marinum* infection. Future functional analysis of these genes may contribute to understanding the mechanisms of mycobacterial pathogenesis. Since a large group of genes linked to immune responses did not show altered expression in the infected animals, these results suggest specific responses in mycobacterium-induced disease.
© 2004 Elsevier Ltd. All rights reserved.

Keywords: Coronin; Inflammatory chitinase; Matrix metalloproteinase; Microarray; Teleost fish

1. Introduction

Pathogenic mycobacteria, including the causative agents of tuberculosis and leprosy, have a characteristic tendency to establish long-term persistent infections. These infections can manifest as acute or chronic disease or can remain latent for many years before progression to clinical disease (Cosma et al., 2003). The precise outcome of an infection depends on complex mycobacterium–host cell interactions which are poorly understood. A hallmark feature of chronic *Mycobacterium tuberculosis* infection is the formation of granulomas, which are organized structures that comprise differentiated macrophages, lymphocytes, other immune cells and extracellular matrix components (Cosma et al., 2003). Mycobacteria can persist intracellularly in macrophages and

---

in necrotic (caseous) areas that can form in the center of granulomas.

Our current understanding of immune responses to *M. tuberculosis* infections is primarily based on the use of cultured cells and a mouse infection model. However, two drawbacks of the mouse model are that *M. tuberculosis* is not a natural pathogen of mouse and that the tuberculosis disease shows a different progression than in human hosts. Particularly, granulomas in mouse do not form as organized structures with a caseous center, a key feature of human granulomas (Pozos and Ramakrishan, 2004). As an alternative, *Mycobacterium marinum* is increasingly used as a model to study mycobacterial pathogenesis. *M. marinum* is the closest relative of the members of the *M. tuberculosis* complex (Rogall et al., 1990). Its natural hosts include various ectotherms, including the leopard frog (*Rana pipiens*), the goldfish (*Carassius auratus*) and the zebrafish (*Danio rerio*), three species for which infection models have been developed (Ramakrishnan et al., 1997; Talaat et al., 1998; Davis et al., 2002; Prouty et al., 2003; Van der Sar et al., 2004a). Among these, the *M. marinum*–zebrafish infection model has the greatest potential to advance insights in mycobacterium–host interactions due to its suitability for genetics studies and for microscopic real-time infection analysis (Pozos and Ramakrishan, 2004; Trede et al., 2004; Van der Sar et al., 2004b).

The pathogenesis of *M. marinum* in zebrafish resembles that of human tuberculosis. Like *M. tuberculosis*, *M. marinum* can survive and replicate within macrophages and has the ability to establish an acute lethal or a chronic progressive disease, dependent on the inoculum and the type of infecting strain (Davis et al., 2002; Prouty et al., 2003; Van der Sar et al., 2004a). Zebrafish granulomas display a similar organization with caseous centers as human granulomas caused by *M. tuberculosis* or by a *M. marinum* skin infection (Prouty et al., 2003; Pozos and Ramakrishan, 2004). The zebrafish is a useful model to study human diseases because it has a complex innate and adaptive immune system with strong similarity to that of other vertebrates (Trede et al., 2004). Homologues of human Toll-like receptor genes that have been implicated in the innate immune response to mycobacterial infections are present in zebrafish and their expression is upregulated in response to *M. marinum* infection (Meijer et al., 2004). A real-time visualization study exploiting the transparency of zebrafish embryos has recently shed light on the importance of innate immune functions for the initial steps in granuloma development (Davis et al., 2002). Furthermore, *M. marinum* infection experiments in leopard frog and zebrafish models have revealed previously unknown aspects of mycobacterium–host interactions by showing that superinfecting mycobacteria rapidly home to pre-established granulomas, where they adapt quickly to escape host defense (Cosma et al., 2004).

Microarray transcriptome analysis is a powerful tool to expand knowledge of the molecular basis of host–pathogen interactions. Available genome sequences from different mycobacteria and several model hosts enable global analysis of transcriptional responses both on the side of the pathogen and on the side of the host. This may lead to the identification of novel factors involved in resistance to mycobacteria and pinpoint targets for therapy development. Not surprisingly therefore, a number of recent studies have applied microarrays to determine mycobacterium-induced expression profiles of different host cell types, including macrophages (Ehrt et al., 2001; Ragno et al., 2001; Nau et al., 2002; Chaussabel et al., 2003; Danelishvili et al., 2003; Nau et al., 2003; Shi et al., 2003; Wang et al., 2003), dendritic cells (Chaussabel et al., 2003), alveolar epithelial cells (Danelishvili et al., 2003), peripheral blood mononuclear cells (Coussens et al., 2003) and effector CD4+ and CD8+ T cells (Cliff et al., 2004). Furthermore, global expression profiles of *M. marinum* genes in frog granulomas (Chan et al., 2002) and microarray patterns of *M. tuberculosis* genes during early infection of mouse (Talaat et al., 2004) have been described.

Here, we report on the expression profile of adult zebrafish showing typical signs of fish tuberculosis due to a chronic progressive infection with *M. marinum*. In the context of this study, a comparative analysis of three types of microarrays was carried out, including MWG and Sigma zebrafish oligonucleotide arrays and the recently marketed Affymetrix zebrafish chip. We present a reference set of 66 upregulated and 93 downregulated genes that consistently showed altered expression in each of the three microarray types. Furthermore, we analyzed the separate datasets of the three types of microarrays with special emphasis on the expression profiles of immune-related genes. Upregulated genes include many known components of the inflammatory response, several genes that have previously been implicated in the response to mycobacterial infections, and a number of genes with unknown relationship to the immune response, whose future functional characterization may contribute to understanding the mechanisms of mycobacterial pathogenesis.

2. Materials and methods

2.1. Zebrafish infection experiments

Adult male zebrafish were infected by intraperitoneal inoculation with *M. marinum* as previously described (Meijer et al., 2004). The fish were sacrificed after 8 weeks, when they showed clear signs of fish tuberculosis with moribund behavior. Histological examination confirmed that the pathology corresponded to fish tuberculosis (Van der Sar et al., 2004a). Control fish were inoculated with phosphate-buffered saline and sacrificed at the same time as the infected fish. Histological examination did not detect any characteristic of fish tuberculosis in control fish.

2.2. Experimental design

For microarray analysis two healthy control fish (c1 and c2) were compared with two fish infected with *M. mar-*