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Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Immunology 42 (2005) 1185–1203
Molecular Immunology
www.elsevier.com/locate/molimm

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*

Received 27 October 2004
Available online 12 January 2005

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.

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

Abbreviations: EST, expressed sequence tag; MHC, major histocompatibility class; RT-PCR, reverse transcriptase-PCR; qPCR, quantitative reverse transcriptase-PCR; TLR, Toll-like receptor

☆ The supplementary tables can also be found at http://zebrafish.liacs.nl/supplements.html.

* Corresponding author. Tel.: +31 71 5274927; fax: +31 71 5274999.
E-mail address: meijer@rulbim.leidenuniv.nl (A.H. Meijer).

0161-5890/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.molimm.2004.11.014

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-*

inum strain M (Ramakrishnan and Falkow, 1994) or E11 (Puttinaowarat et al., 2000) (fish i1 and i2, respectively). RNA samples from the same fish were previously used to demonstrate that the expression of several Toll-like receptor genes was upregulated in mycobacterium-infected fish compared with control fish (Meijer et al., 2004). For the oligonucleotide microarrays, dual-color hybridizations were performed with RNA probes from fish c1 (Cy3) versus i1 (Cy5) and from fish c2 (Cy5) versus i2 (Cy3). Both comparisons were performed in duplicate, resulting in a total of four datasets for the MWG array and four datasets for the Sigma array. To check for consistency within the spotted oligonucleotide microarrays, Ambion spikes and oligo test sets (see below) were compared. For the Affymetrix analysis, two GeneChips were used for the control fish and two GeneChips for the infected fish.

2.3. RNA preparation

Total RNA samples were isolated from zebrafish homogenized in liquid nitrogen and extracted using TRIZOL reagent (Invitrogen) according to the manufacturer's instructions. Next the RNA samples were purified over RNeasy columns (Qiagen) according to the RNA Cleanup protocol in the RNeasy Mini Handbook (Qiagen), including the on-column DNAse digestion step. Concentrations of purified RNA samples were determined by A_{260} measurement and the quality was checked by Lab-on-a-Chip analysis (total RNA nano biosizing assay, Agilent) with the Agilent 2100 Bioanalyzer.

2.4. Production and hybridization of oligonucleotide microarrays

Microarrays of the MWG 14k Zebrafish Oligo Set and the Sigma-Genosys 16k Zebrafish OligoLibrary were produced using the facilities of the Leiden Genome Technology Center (http://www.lgtc.nl/). Oligonucleotides were dissolved in 150 mM phosphate buffer (pH 8.5) to a concentration of 20 μM and spotted on poly-L-lysine-coated glass slides or on CodeLink Activated Slides (Amersham Biosciences) as described in ('t Hoen et al., 2003). Ambion's ArrayControl oligos (eight sense oligos) and three custom-designed MyD88 oligonucleotides were spotted together with the MWG Oligo Set. In addition, the Sigma zebrafish test set (ZEBLIBTST96) and Sigma zebrafish set XEB384-15 were added to the MWG set to allow us to check for consistency with results from the Sigma microarrays. Spotted CodeLink Activated Slides were treated with Blocking Solution and washed according to the manufacturer's instructions.

Total RNA (2 μg) was amplified using Ambion's MessageAmp kit by ServiceXS (Leiden, The Netherlands). During in vitro transcription, 5-(3-aminoallyl)-UTP (Ambion) was incorporated. Subsequently, 5 μg of cRNA was used for coupling of Amersham's monoreactive Cy3 or Cy5 dyes and 1.5 μg of the fluorescent labeled cRNA was used for the hybridizations as described in 't Hoen et al. (2003). Ambion's ArrayControl RNA spikes (amount per spike varying from 0 to 200 pg) were added during the labeling reaction. Labeled cRNA was purified over RNeasy mini columns (Qiagen) and the quality was checked by Agilent's Lab-on-a-Chip total RNA nano biosizing assay. Prehybridization, hybridization (55 °C) and scanning were carried out as described in 't Hoen et al. (2003).

2.5. Hybridization of Affymetrix microarrays

Total RNA (10 μg) was used for first and second strand cDNA synthesis as described in the Affymetrix GeneChip Expression Analysis Technical Manual. The resulting double-stranded cDNA was used as a template for biotin labeling with the MEGAscript T7 kit from Ambion according to the manufacturer's instructions. Hybridization and scanning were performed according to standard Affymetrix protocols. Equal amounts of labeled cRNA (10 μg) were used per zebrafish GeneChip array. The 3′/5′ ratio values for GADPH were below the acceptable level of 2 in all experiments. The number of present calls varied between 72 and 75%.

2.6. Data analysis

For spotted oligonucleotide arrays, individual feature intensities were extracted from scanned microarray images using GenePix Pro 5.1 software (Axon Technologies). Affymetrix GeneChip data were extracted and normalized using Affymetrix GCOS software. Data outputs were imported into Rosetta Resolver 4.0 (Rosetta Inpharmatics LLC). Individual arrays were normalized using default settings. After that, replicate ratio results from each technology were combined using the default ratio experiment builder implemented in the Rosetta Resolver system (for details see http://info.rosettabio.com/). In both types of arrays (ratio and intensity), infected fishes were compared against control fishes. For the dataset shown in Fig. 2, an extra selection criterion was used based on GenePix data normalization on β-actin signals. For construction of the reference set shown in Table 3, the datasets were linked to the UniGene cluster assignments, which were updated according to the Dr.gb_cid_lid datafile (10 July 2004) supplied by NCBI. The separate datafiles from the microarray experiments were imported in a relational database (MS-Access). For each of the array experiments, a separate table was made. The EST GenBank accession codes and the UniGene codes were used to produce joins between the separate tables. Filter queries on the tables reduced the number of data in the final analysis. Frequency analysis on the data revealed the number of UniGene clusters as listed in Table 1.

2.7. Quantitative RT-PCR

Quantitative RT-PCR (qPCR) was performed using the LightCycler Instrument with the LightCycler RNA Master SYBR Green I kit (Roche). Amplicons were generated dur-

ing 45 cycles with the annealing temperature set at 55 °C and cycle parameters as recommended in the instruction manual. RT-PCR mixtures contained 1–100 ng total RNA, 3.25 mM $Mn(OAc)_2$ and 0.5 μM forward and reverse primer in 20 μl of 1× RNA Master SYBR Green I mix. RNA samples were identical to those used for microarray hybridization, providing duplicate measurements for the control and infected fish. Based on a dilution series, 10 ng of RNA was taken for amplification of complement factor C3 and 100 ng for amplification of the other genes. Data were normalized using a standard curve for β-actin amplification on 1, 3, 10 and 100 ng RNA. β-Actin showed unchanged expression between control and infected fish based on both qPCR and microarray data. Melting curve analysis was performed to verify that no primer dimers were amplified. Sequences of forward and reverse primers were 5′-CGAGCAGGAGATGGGAACC and 5′-CAACGGAAACGCTCATTGC for β-actin (AF057040), 5′-CCCAGCTGTCATCAACTCTG and 5′-TTACGTGGTCCAAGCACATC for gelatinase B (matrix metalloproteinase 9, AW174507), 5′-AATACCCCTGGCTAGCAAAG and 5′-GTGTGTCATCAACCCTGAAG for complement factor B (AI601749), 5′-CTTACAACTTCCCGACTGTC and 5′-AGTTGGATGCCACAGAACTG for vertebrate coagulation factor v and vii homolog (AL590146_3, wz14055.1), 5′-GTCGTCCAAGTTGCAAAGAG and 5′-CAGGTCTGCTCTCCCAAGA for complement factor C3 (BM095286), 5′-CTACTCGCTGACTGGAGAAC and 5′-CCAGACCCATGAATCCACTC for cathepsin D (NM_131710), and 5′-CTCCTCGGAGTTCACCTTG and 5′-GTTGACTTGCATCCCATCTAC for major histocompatibility class I UFA gene (AF137534).

3. Results

3.1. Comparative analysis of Affymetrix, MWG and Sigma microarray data

In this study, we examined the gene expression profile of adult zebrafish at the late stage of a chronic progressive form of fish tuberculosis caused by two *M. marinum* strains. Transcriptome analyses were performed using microarrays of the MWG Zebrafish Oligo Set, the Sigma Zebrafish OligoLibrary and the Affymetrix GeneChip Zebrafish Genome Array (see Table 1 for detailed characteristics). The resulting datasets were analyzed using Rosetta Resolver. The numbers of differentially expressed genes detected at different *P*-values are shown in Fig. 1. At a significance level of $P < 1.00E{-}5$, the total numbers of more than 2-fold upregulated (Affymetrix: 386; MWG: 203; Sigma: 287) or downregulated (Affymetrix: 265; MWG: 260; Sigma: 295) genes were in a similar range. We concentrated further on subsets of genes with upregulated expression according to strict selection criteria for technical and biological duplicates explained in the legend of Fig. 2. This figure shows that the distribution of the upregulated genes over different functional categories was remarkably similar. The induced sets from the Affymetrix and Sigma microarrays gave an almost identical distribution picture, while the set from MWG contained somewhat less genes in the category of genes involved in signal perception and transduction and contained a larger category of metabolism genes. The larger metabolism category in MWG was mainly due to the abundant presence of ferritin H subunit genes belonging to the same UniGene cluster, which comprised 40% of this category. The sets of induced genes also included many genes that we could not assign to a functional category because our BLAST searches with the corresponding ESTs had not detected significant homology to proteins with known function. The Affymetrix set of induced genes consisted for 37% of such unannotated genes, the Sigma set for 46%, but the MWG set for only 5%. This might reflect the different assembly methods used in the different microarray platforms.

Examples from the different categories of induced genes are listed in Table 2. The group of carriers and transporters contained a large number of vacuolar proton-pump ATPases, involved in lysosome acidification. A group of different coagulation factors was also induced, which is not surprising because the chronically ill zebrafish showed skin lesions. Indicative of the severe disease stage, we observed induction of many genes with roles in cytoskeleton structure and dynamics or in membrane function as well as induction of genes encoding components of the extracellular matrix. The group of induced metabolic genes encoded enzymes acting on the extracellular matrix, lysosomal enzymes, proteins involved in

Table 1
Overview of the zebrafish microarrays

	Affymetrix	MWG	Sigma
(A) Characteristics of the zebrafish microarrays			
Product name	GeneChip 430	Zebrafish 14k	Sigma-Compugen XEB384
Oligo length	25	50	65
# Oligos	15502 (×16)	14067	16399
# Oligos with UniGene assignment	14953	8669	12662
# Unique UniGene clusters	12525	5416	11166

	Affymetrix–MWG	Affymetrix–Sigma	MWG–Sigma
(B) Pair-wise comparisons between the zebrafish microarrays			
Overlap in identical ESTs	1321	4207	1324
Overlap in unique UniGene clusters	4689	9221	4576

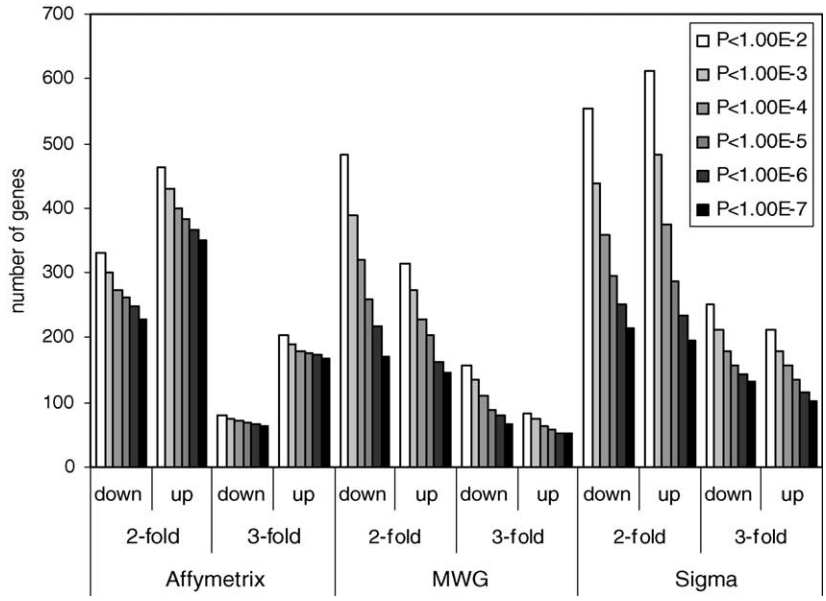

Fig. 1. Significance of the microarray data. The graph displays the number of genes on Affymetrix, MWG and Sigma microarrays that showed ≥2- or ≥3-fold upregulated or downregulated expression in response to *M. marinum* infection at different *P*-values determined by Rosetta Resolver analysis.

iron homeostasis and a variety of other metabolic enzymes. An induced group of genes involved in DNA/RNA synthesis, processing or degradation was also observed, among which the apoptosis-specific endonuclease, DNAse gamma. Furthermore, the apoptosis-specific protease, caspase a (Inohara and Nunez, 2000), was present in the category of genes involved in protein synthesis, processing or degradation. This category also included other proteolytic enzymes, such as cathepsins, implicated in antigen presentation and tissue remodeling (Turk et al., 2002) and matrix metalloproteinases, also implicated in remodeling of the extracellular matrix and in migration of myeloid cells (Parks et al., 2004). A group of induced stress and defense response genes included several genes specific for myeloid cells, such as the myeloid-specific peroxidase, components of the phagocyte oxidase system and lysozyme C. Finally, we could distinguish four other categories of genes whose function can be linked to the immune response or its regulation. These categories, which will be discussed further below, comprised the complement activation and acute phase response genes, genes related to the adaptive immune response, genes involved in signal perception and transduction, and genes encoding transcription factors.

3.2. A reference set of zebrafish genes responsive to M. marinum infection

To determine the precise overlap between the results from Affymetrix, MWG and Sigma microarrays, the Rosetta Resolver datasets were linked based on the UniGene cluster assignments. Table 1 shows the overlap in identical ESTs and UniGene clusters that exists between the three different platforms. The total overlap between the three microarray types comprises 4138 different UniGene Clusters, in most cases consisting of multiple ESTs. This linked dataset was analyzed for genes showing increased or decreased expression in the *M. marinum*-infected fish compared to the controls. Table 3includes genes that demonstrated at least 2-fold difference in expression based on the data from all the different microarray types. Therefore, this list, representing 66 upregulated and 93 downregulated UniGene clusters, serves as a highly trustworthy reference set for transcriptome analysis in the *M. marinum*–zebrafish infection model. A criterion for this reference set was that the differential expression should occur with both of the *M. marinum* strains (M and E11) that were used in this study. Less stringent selection criteria (e.g. allowing 1.5-fold difference instead of 2-fold) indicated that a much larger overlap between the positively and negatively regulated datasets is present, which can be derived from supplementary Table S7. Furthermore, the reference set can be increased considerably when the MWG data are not included, since the MWG library contained approximately 50% less oligonucleotides that could be assigned to different UniGene clusters compared with the Affymetrix and Sigma libraries (Table 1).

The 66 upregulated genes in the reference set included representatives from almost all the functional categories that we could previously distinguish in the analyses of the separate datasets. For example, the category of carriers and transporters again included several different vacuolar proton-pump ATPases. However, not all genes from this category were present in the reference set. This underscores the usefulness of analyzing different microarrays. For instance, an ABC transporter gene was identified only from analysis of the Sigma oligonucleotide set. Other functional categories of the reference set (such as those of the coagulation factors,

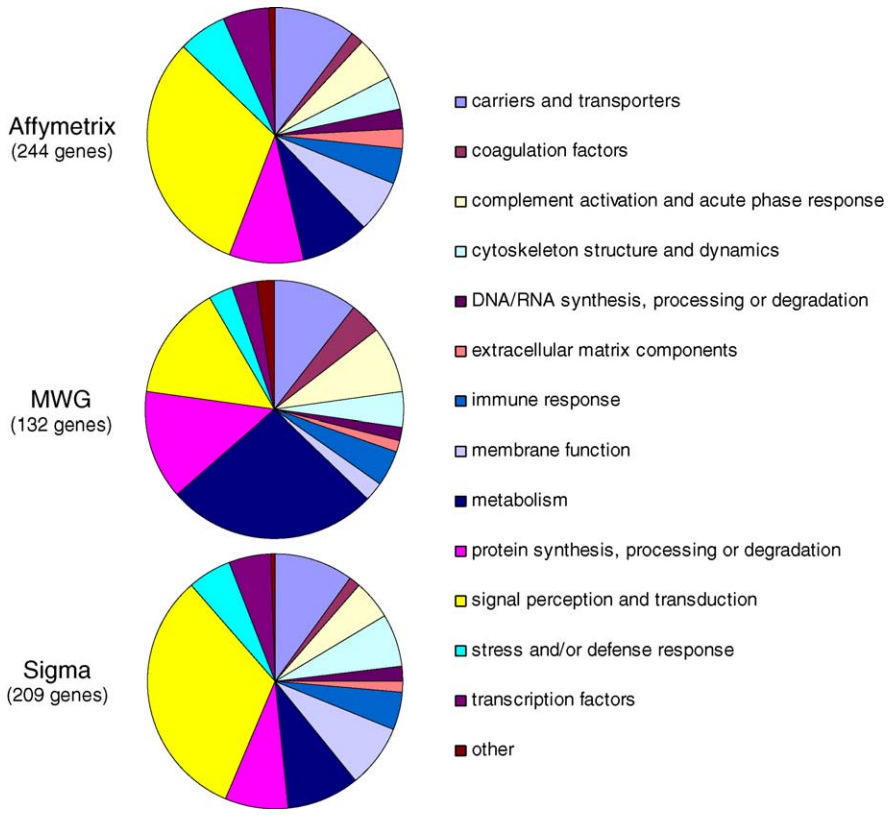

Fig. 2. Distribution of infection-induced genes over different categories.The Affymetrix diagram is based on genes showing ≥2-fold induction at $P < 1.00E{-}5$ according to the Rosetta analysis (see Fig. 1). For construction of the MWG and Sigma diagrams, the Rosetta dataset of ≥2-fold induced genes at $P < 1.00E{-}3$ was subjected to a second data analysis using GenePix 5.1 software with normalization on β-actin signals. Genes showing ≤2-fold induction by the second method (Sigma: 19%; MWG: 42%) were deleted from the Rosetta dataset. We chose to use the two different normalization methods (the Rosetta normalization algorithm is based on all array signals) to increase the validity of the dual-color hybridization results. A criterion implemented in both the Rosetta and GenePix analysis was that differential expression should be detected after infection with the *M. marinum* M strain as well as the E11 strain. Genes showing no significant homology to known proteins or homology to hypothetical proteins with unknown function are not shown in the pie-diagrams (Affymetrix: 142 genes; MWG: 7 genes; Sigma: 181 genes). Examples from the different categories of upregulated genes are listed in Table 2. Gene descriptions and microarray data underlying the pie-diagrams are given in supplementary tables S1 (Affymetrix), S2 (MWG) and S3 (Sigma). Supplementary tables S4 (Affymetrix), S5 (MWG) and S6 (Sigma) contain the datasets of genes with downregulated expression according to the same criteria.

the extracellular matrix components, the membrane function genes and the DNA and RNA synthesis/metabolism genes) also showed less variety than was detected in the analysis of the separate datasets. On the other hand, most representatives from the category of genes with functions in cytoskeleton dynamics were found back in the reference set. These included L-plastin, a macrophage-specific actin-bundling protein (Herbomel et al., 1999), coronin, an actin-binding protein associated with the mycobacterium phagosome (Ferrari et al., 1999), and members of the arp2/3 complex involved in actin polymerization. Furthermore, the reference set also contained a variety of proteolytic enzymes, including cathepsins, matrix metalloproteinases and caspase a. Other categories in the reference set contained several genes which are of interest in relation to the immune response. These include complement activation and acute phase response genes that are abundantly represented in the reference set. The group of stress and defense genes included the tartrate-resistant acid phosphatase 5, which is a lysosomal and secreted glycoprotein produced by macrophages and osteoclasts (Bune et al., 2001). The metabolism group included ferritin H, a protein involved in iron homeostasis. However, ferritin H might also fit in the signal transduction category, since its interaction with the granulocyte colony-stimulating factor receptor was recently proposed to trigger a signaling pathway regulating the formation of reactive oxygen species in neutrophilic granulocytes (Yuan et al., 2004). While the analyses of the Affymetrix, MWG and Sigma microarray had identified several induced B- and T-cell-specific proteins that we grouped under immune response genes, this was the only category that was not found back in the reference set. However, the signal perception and transduction and transcription factor categories contained several other genes related to the immune response, as will be discussed below.

The set of genes with decreased expression in the mycobacterium-infected fish is relatively large. Repression was observed of a large number of muscle-specific proteins and many metabolic enzymes, different keratins and several retinal proteins. With respect to defense responses notably the expression of MHC class 1 proteins and of a mannose-binding

Table 2
Selection of zebrafish genes with well defined functional annotation induced by *M. marinum* infection

Carriers and transporters
- ABC transporter (ABC1) (S)
- Lysosomal H^+-transporting ATPase (various subunits) (A, M, S)
- Solute carrier family proteins (A, M, S)

Coagulation factors
- Coagulation factors v and viii (A, M)
- Fibrinogen (M, S)

Complement activation and acute phase response
- Alpha-1-antichymotrypsine (S)
- Alpha-1/alpha-2-macroglobulin (S/A, M)
- Complement-activating component of Ra-reactive factor (A, M, S)
- Complement components C3a (M, S)
- Complement component C4a (A, M)
- Complement component C6 (S)
- Complement component C7 (A)
- Complement component C9 (A)
- Complement factor B (M, S)
- Haptoglobin (A)
- Properdin P factor (S)
- Serum amyloid protein (A, M)

Cytoskeleton structure and dynamics
- Coronin (A, M, S)
- Dynein (A, S)
- LIS1-interacting protein NUDEL (S)
- L-plastin (A, M, S)
- Moesin (msvn) (A, S)
- Microtubule-associated protein EB1 (M)
- p41-arc (arp2/3 subunit 1b) (A, M, S)
- Talin (tln) (S)
- Tubulin α6 (M)
- WASP (A, S)

Extracellular matrix components
- Collagen α1 (A, M, S)
- Fibronectin 1 (A)
- Fibulin-4 (S)
- Microfibril-associated glycoprotein 4 (A, M)
- Thrombospondin 1 (A)

Immune response
- Epidermal growth factor-response factor 1 (A, S)
- Galectin 9 (A)
- Glucocorticoid-sensitive T-cell-specific protein (S)
- Immediate early gene IEX-1 (S)
- Immunogulobulin heavy chains (A)
- Immunoglobulin light chains (A, M, S)
- Lymphocyte antigen 116 (A, M, S)
- Novel immune-type receptor 1.8 (nitr1.8) (M)
- Nucleolysin TIAR (A, S)
- Osteopontin (secreted phosphoprotein 1, ssp1) (A)
- Protein Rei, Bence-Jones (S)
- Secretory granule proteoglycan core protein (A, S)
- Tumor necrosis factor alpha-induced protein 2 (S)

Membrane function
- CD63 antigen (LAMP-3) (A, S)
- Flotillin-2 (Epidermal surface antigen) (ESA) (A, S)
- Hematopoietic protein 1 (HEM1) (S)
- Leukocyte surface antigen CD53 (A)
- Lysosome-associated membrane glycoprotein 1 (LAMP-1) (A, S)

Table 2 *(Continued)*
- P-selectin precursor (LECAM3) (S)
- Syntaxin 11 (t-SNARE) (A, S)
- Transmembrane 4 superfamily proteins (A, S)
- Transmembrane 7 superfamily proteins (A, S)

Metabolism
- Cholesterol 24-hydroxylase (cytochrome P450 46) (A)
- Ferritin H subunit (A, M, S)
- Glyceraldehydes 3-phosphate dehydrogenase (M)
- Glutathione reductase (A, S)
- Lysosomal phospholipase A2 (A)
- Procollagen-lysine 5-dioxygenase (S)
- Sphingomyelin phosphodiesterase 1, acid lysosomal (S)
- Thioredoxin (S)

DNA/RNA synthesis, processing or degradation
- Cleavage and polyadenylation specific factor 1 (M)
- Deoxyribonuclease gamma precursor (DNase gamma) (S)
- Ribonuclease 6 precursor (S)

Protein synthesis, processing or degradation
- Caspase a (A, M, S)
- Cathepsins (different types) (A, M, S)
- Cystatin F (leukocystatin) (S)
- Eukaryotic translation initiation factor 4E binding protein 3 (M)
- Histone deacetylase 9 (hdac9) (A)
- Matrix metalloproteinase 9 (gelatinase-B) (A, S)
- Matrix metalloproteinase 13 (collagenase 3) (M, S)
- Matrix metalloproteinase 14α (mmp14a) (A, S)
- Metalloproteinase inhibitor 2 (A)
- Nephrosin (astacin metalloproteinase) (A)
- Protease nexin I (M)
- 26S protease regulatory subunits (A, M, S)

Signal perception and transduction
- Chemokine receptor 4b (cxcr4b) (A, M, S)
- c-IAP1 (TNFR2-associated) protein (A, M)
- Cyclin-dependent kinase 5 activator protein (S)
- Cytokinase-inducible kinase (proliferation-related kinase) (A)
- Cytokine-inducible SH2 protein 3 (SOCS3) (A, S)
- Delta C (A)
- GABA(A) receptor-associated proteins (A, S)
- Progranulin (PC cell-derived growth factor) (A, M, S)
- Hematopoietic lineage cell-specific protein HS1 (S)
- Hematopoietic cell-associated (GRB2-related) adaptor (A)
- Insulin-like growth factor binding protein 1 (A, M)
- Interleukin-1 receptor-associated kinase 4 (IRAK4) (A, M)
- Interleukin-13 receptor, α2 (A)
- Integrin α5 subunit (S)
- Janus kinase 1 (JAK1) (A, M, S)
- Mitogen-activated protein kinase kinase kinase 14 (A, S)
- NFκB inhibitor alpha-like protein A (ikbaA) (A, S)
- Notch receptor homologs (A, M, S)
- p38β (mitogen-activated protein kinase 11) (A)
- Phospholipid scramblase 1 (A, M, S)
- Protein-glutamine gamma glutamyltransferase (A)
- Proto-oncogene serine/threonine-protein kinase pim-1 (A, S)
- Protein tyrosine phosphatase receptor CD45 (A)
- Rac2 GTP-binding protein (M)
- Ras-related protein Rab-7 (S)
- Rho guanine nucleotide exchange factors (A, S)
- Semaphorin protein receptor, plexin D (A)
- Signal-transducing adaptor molecule 1 (stam) (S)
- Smad ubiquitination regulatory factor 1 (SMURF1) (S)
- Sorcin (A, M, S)

Table 2 *(Continued)*

SRC-kinase associated phosphoprotein (S)
Syndecan-binding protein (syntenin) (A, M)
Switch-associated protein (SWAP) (A)
Urokinase plasminogen activator surface receptor (uPAR, CD87) (A)
Stress and/or defense response
Chitotriosidase gp39c (eosinophil chemotractic factor-L) (A, M)
Five-lipoxygenase-activating protein (FLAP) (A, S)
Lysozyme C (A)
Neutrophil cytosolic factor 1 (p47-phox) (A, S)
Neutrophil cytosolic factor 4 (p40-phox) (A, S)
Hydroxyacid oxidase 1 (glycolate oxidase) (S)
Myeloid-specific peroxidase (mpx) (A)
Prostaglandin I2 (prostacyclin) synthase (A, S)
Selenoprotein P, plasma, 1b (sepp1b) (A)
Tartrate-resistant acid phosphatase 5 precursor (A, M, S)
Tumor suppressor pHyde (A, S)
Transcription factors
B-cell CLL/lymphoma 5 (BCL5) protein (S)
CCAAT/enhancer binding protein (C/EBP) alpha (S)
CCAAT/enhancer binding protein (C/EBP) beta (A, M, S)
Ikaros (lymphoid transcription factor LyF-1) (A)
LPS-induced TNFα factor (LITAF) (A)
Neurogenin 1 (M)
Spi1 (PU.1) (A, M, S)

Examples are shown from different categories of the infection-induced genes that are reported in Fig. 2. Genes annotated by GenBank with known relevance to the response to infection have been selected in particular. The complete lists of induced genes and the GenBank accession numbers are reported in supplementary tables S1 (Affymetrix), S2 (MWG) and S3 (Sigma). Gene descriptions are based on significant homology with human and mouse genes and in some cases have been confirmed by functional studies in zebrafish. The genes were grouped in different categories based on their functions described in the literature or on putative functions inferred from sequence homology to genes with known functions. The source of the data (Affymetrix (A), MWG (M) and/or Sigma (S) microarray experiments) is indicated between brackets.

lectin is repressed. Two cell adhesion molecules, claudin 10 and cadherin-17, were also downregulated.

3.3. Immunity-related genes in the Affymetrix, MWG and Sigma datasets

Both the reference set and the datasets from the separate platforms were further analyzed for the presence of upregulated genes that could be functionally related with the immune response to mycobacterium infection. Increased expression of a number of different complement factors and acute phase response proteins (e.g. serum amyloid protein and alpha-1-macroglobulin) was indicative of an activated innate immune response in the infected fish. Evidence of an adaptive immune response was apparent from the induction of several immunoglobulin genes and different T-cell-specific proteins. We also observed induction of the homologs of different immune response genes, such as the TNFα-induced protein 2 gene. Furthermore, a member of the novel immune type receptor (Nitr) family was induced. Nitr receptors, which have been found in different species of teleost fish, contain variable regions resembling prototypic forms of immunoglobulin and T-cell antigen receptors and are thought to represent intermediates in the evolution of the leukocyte receptor cluster (Litman et al., 2001).

The induced sets of genes from all platforms and the reference set contained many genes involved in signal perception and transduction. Among these were several immunity-related receptors, including a chemokine receptor (cxcr4b), an interleukin receptor homolog, a cell adhesion receptor (integrin α5) and notch receptor homologs. Notch signaling is required for T-cell development and the Notch-ligand delta C was also identified among the induced genes. Many upregulated genes encoded homologs of signal transduction proteins with known immune relation. A few examples are Janus kinase 1 (JAK1), involved in cytokine signaling, SOCS3, a Janus kinase inhibitor, Skap55, a PH-and SH3-domain-containing adaptor protein involved in T-cell receptor activation (Wu et al., 2002), and the pim1 oncogene, encoding a non-receptor serine/threonine kinase linked to hematopoietic development (Icard-Liepkalns et al., 1999). Growth factors were also induced, including the PC cell-derived growth factor, progranulin, a probable mediator of the wound response (He et al., 2003). Another interesting induced signal transduction mediator was phospholipid scramblase 1, a component of membrane lipid rafts which has been shown to interact with raft-associated cell surface receptors and is involved in differentiation of hematopoietic precursors (Nanjundan et al., 2003).

A number of transcription factor genes were induced of which functions in hematopoiesis or in regulation of the immune response have been described. Three of these, Spi (PU.1), c/ebpα and c/ebpβ, are known to be expressed in embryonic myeloid cells of the zebrafish (Lyons et al., 2001a; Crowhurst et al., 2002). Spi (PU.1) is a member of the ETS family of transcription factors that is required for the development of multiple hematopoietic lineages. Members of the C/EBP (CCAAT enhancer binding protein) family comprise key regulators of cellular differentiation and the inflammatory response in mammals (Lekstrom-Himes and Xanthopoulos, 1998). Other induced transcription factor genes included homologs of Ikaros, an important regulator of lymphocyte differentiation (Georgopoulos, 2002), the LPS-induced TNFα factor (LITAF), which has a presumed role in the regulation of TNFα gene expression (Bolcato-Bellemin et al., 2004), and the glucocorticoid-induced leucine zipper protein (GILZ), a modulator of T-cell activation and apoptosis (Ayroldi et al., 2001).

The most obvious immune-related genes present in the datasets of downregulated genes were the MHC class I genes, which were also represented in the reference set (Table 3). In addition, various MHC class II genes were also found to be downregulated in each of the individual datasets (see supplementary Tables S4–S6).

Although the microarray analyses detected differential expression of many genes with relevance to the immune response (Tables 2 and 3), several other important genes were notably missing from the lists of differentially expressed

Table 3
Reference set of zebrafish genes responsive to *M. marinum* infection

UniGene	GenBank accession numbers	Fold difference			
		Affymetrix	MWG	Sigma	Description
(A) Upregulated genes					
Carriers and transporters					
Dr.14783	BC053214, BI888972, AI959690	2.8	2.1	2.7	H^+-transporting ATPase, lysosomal (atp6v1c1)
Dr.26356	AL721636, BI473941, BE693186	100.0	2.6	20.7	Hemoglobin alpha embryonic-1 (hbae1)
Dr.2855	AI958585, BM534347, BM530957	3.4	2.1	1.6**	H^+-transporting ATPase (VA68 type)
Dr.370	BI879966, AF210636	2.4	2.1	3.1	H^+-transporting ATPase (atp6v1g1)
Dr.6504	NM_153663, AF465772, BI888455	1.9	2.1	2.6	Spinster-like sugar transporter protein
Dr.7822	BQ264141, AW233763	2.1	2.7	2.2	H^+-transporting ATPase, lysosomal accessory protein 2
Coagulation factors					
Dr.845	BG729013, BG303403, BM181145, AW342845	1.7	2.7	3.2	Fibrinogen alpha/alpha-E chain precursor
Dr.8505	BI878927, BQ264051	1.7	2.8	2.5	Fibrinogen, B beta polypeptide (fgb)
Complement activation and acute phase response					
Dr.12491	BI672168	2.1	2.8	2.0	Complement C4A precursor
Dr.13131	BI883568, BQ131518	22.7	8.4	6.8	Serum amyloid A3/A2-beta protein precursor
Dr.190	NM_131338, U34662	1.8	2.5	2.4	Complement component factor B
Dr.28414	BI474808, BI889778, AI957415, AI545973, BI326782, BI476861, BM530427	2.4	2.5	2.1	Alpha-1-macroglobulin
Dr.4216	AI477496, AI496785	6.7	2.1	3.8	Complement-activating component of Ra-reactive factor
Cytoskeleton structure and dynamics					
Dr.7105	BC053229, AI958799, CA471002, AW232279	2.9	2.2	2.2	Actin-related protein 2/3 complex, subunit 1B (arpc1b)
Dr.8109	AF157110, BI889183	3.5	2.6	2.7	L-plastin
Dr.8744	CD598194, AI353994, AW154456, AI878656	2.4	1.9	2.2	Actin-related protein 2
Dr.9515	BM095940	3.1	3.3	2.9	Coronin
Dr.9089	BC046879, BI891788, AW203156	3.2	1.6	2.8	Retinitis pigmentosa 2 (XRP2)
DNA/RNA synthesis, processing or degradation					
Dr.5122	AW567501, CA476057, BI847074	4.4	1.9*	1.7**	Heterogeneous nuclear ribonucleoprotein A0
Extracellular matrix components					
Dr.11427	BG302899, CA473065	2.4	1.9	2.3*	Protein-lysine 6-oxidase precursor
Dr.18835	BQ450149/BF717540, BF717537	68.4/2.3	2.9	3.3*	Microfibril-associated glycoprotein 4
Membrane function					
Dr.22797	AW281815, AW281510	3.4	2.5	3.0	Lysosomal-associated multitransmembrane protein
Metabolism					
Dr.16208	BG883755, BI473433, BG799846	9.1	6.4	5.2	Cytidine deaminase
Dr.18396	BC049493, CA472740, BI892352	2.2	2.6	3.3	Thioredoxin interacting protein (txnip)
Dr.23977	CD014982, AW233107, AW116095	23.5	3.5	3.3	NADP-dependent malic enzyme (NADP-ME)
Dr.2644	AI626374, AI794200	2.5	1.9	3.0	Dehydrogenase/reductase (SDR family) member 3

Table 3 *(Continued)*

UniGene	GenBank accession numbers	Fold difference			
		Affymetrix	MWG	Sigma	Description
Dr.2783	AI942927, BG306717, BG728460	1.8	2.0	2.3*	Phosphatidylinositol-3-phosphate/phosphatidylinositol 5-kinase
Dr.28742	AW076584, AW077112, AW202929, AW232676, BE201201, BG303615, BG892155, BG985532, BI708028, BI980455, BM141358, BM141472, BM155225, BQ132177, BQ449760, BQ615936, BQ783379	2.1	3.2	3.2	Ferritin H subunit
Dr.3613	BC048037, AF336125	2.5	2.6*	2.3**	Ceruloplasmin
Dr.466	BI890155/CA452365, AW154548	13.0/2.4	2.0**	3.3	Acetyl-CoA synthetase 2-like
Dr.6619	BC044196, BI896925, AW115782	2.2	2.4	2.0	6-Phosphogluconate dehydrogenase
Protein synthesis, processing or degradation					
Dr.10788	NM_131804, AJ278269	24.0	2.4	2.9**	Cathepsin E precursor
Dr.1168	NM_131505, AF233434	2.4	2.8	2.0*	Caspase a (caspa)
Dr.1187	BG305906, AI959324	2.2	2.1**	2.2	26S protease regulatory subunit 4 (P26s4)
Dr.14028	BQ479892, BI707739	4.4	1.6*	2.2	Goliath protein (G1RP)
Dr.19238	NM_131710, AJ278268	2.9	3.5	1.6**	Cathepsin D (ctsd)
Dr.29852	BI475931, AA606011, BQ419101, BM081219	2.7	1.5	2.7	Matrix metalloproteinase 14 (mmp14a)
Dr.4048	AL926284, BQ616186, AI384696	2.7	2.4	2.9	Cathepsin K
Dr.4647	BC046079, AF332983	1.8	2.9	1.9*	eIF4E-binding protein 3
Dr.967	BC053292, AW174507, BI325744	13.3	10.9	12.8	Matrix metalloproteinase 9 (gelatinase B)
Signal perception and transduction					
Dr.10051	BM736017, BE200777, BE556826	2.9	2.0	2.4	Cyclin G2 (ccng2)
Dr.10320	BC052134, CA496502, BI673466	1.8	2.0	3.6	Serum/glucocorticoid regulated kinase (sgk1)
Dr.1278	BC053237, AI877607, BM102105	2.5	2.4	2.4	Sorcin
Dr.12845	BI864156, CA474818, BI882581	2.2	2.3	1.9*	GABA receptor associated protein (Gabarap)
Dr.14593	BC053164, BI890764, AI958597	2.2	1.5	2.9	GTP-binding regulatory protein Gi alpha-1 chain
Dr.16684	BI883263, AW566698, AW154420	2.2	2.4	2.3	Phospholipid scramblase 1
Dr.16985	BI326583, BM026089, BM025857	2.5	2.6	2.6	Protein kinase c-like 2 (prkcl2)
Dr.1778	BC044454, BQ260284, BI886016	2.9	1.7**	2.7	Syndecan-binding protein (syntenin, sdcbp)
Dr.23975	AW116003, AW128034	5.0	3.2	3.9	Ras-like GTP-binding protein Rad
Dr.275	BQ260616, AJ005689, U82980	3.7	1.8	1.7**	Janus kinase 1 (JAK1)
Dr.28244	AI957547, AI416180	1.8	1.7**	3.6	Guanine nucleotide binding protein (G(i) protein, gnai2l)
Dr.2849	AF442500	2.3	2.9	2.5*	Inhibitor of apoptosis protein (iap1)
Dr.32764	AW165158, BI704528	3.2	1.9	3.1	Integrin α5 subunit
Dr.4748	AF273481, AF273479	3.9	9.1	5.1	Granulin
Dr.6431	BC049326, AW421627, BI878700	3.6	1.6**	4.2	Cytokine inducible SH2-containing protein 3 (SOCS3)
Dr.6798	NM_131834, AF201451	5.0	2.3	2.9	Chemokine receptor 4b (cxcr4b)
Dr.7257	AW115682, BM072526	2.8	1.8	2.2	Ras/Rap1A-associating guanine nucleotide exchange factor
Dr.8587	AL910822, NM_173283, AJ299409	2.4	3.1	4.9	Insulin-like growth factor binding protein 1 (igfbp1)
Stress and/or defense response					
Dr.610	NM_131328, AF068773	2.7	2.2	2.2	Heat shock protein hsp90α

Table 3 *(Continued)*

UniGene	GenBank accession numbers	Fold difference			
		Affymetrix	MWG	Sigma	Description
Dr.1508	AI882824, BI673538, CA473818	5.3	3.2	4.4	Tartrate-resistant acid phosphatase 5 precursor
Dr.25730	CD605058, AL912007, BI672496	1.9	2.2**	2.3	Testin
Dr.8516	NM_178298, AF322072	13.1	1.8	3.1**	Selenoprotein P precursor
Transcription factors					
Dr.12437	BQ285072, CA474031, BI846940	1.6	2.1	2.8*	Glucocorticoid-induced leucine zipper protein (GILZ)
Dr.6575	BC049401, NM_131884, AW019436	3.7	4.4	5.4	CCAAT enhancer binding protein (C/EBP) beta
Unknown					
Dr.28005	AW154575, CA472666, BI867521, BI704359	2.4	1.5*	2.6	Unknown
Dr.2899	AI415936, AI384820	2.5	2.0	1.7	Unknown
(B) Downregulated genes					
Antigen presentation					
Dr.21017	BC053140, AF182156, AI477589	−100.0	−3.1	−51.9	MHC class I UEA gene (mhc1uea)
Dr.21018	AF182155	−20.9	−3.0*	−2.2*	MHC class I antigen (Dare-UDA)
Dr.4198	AF137534	−100.0	−36.3	−20.3	MHC class I UFA gene (mhc1ufa)
Carriers and transporters					
Dr.12516	BM095208, AL918579	−2.2	−1.8**	−3.0	CGI-69 mitochondrial carrier protein
Dr.3	BE200552, NM_131221	−4.0	−1.5**	−2.6	Na+/K+ transporting ATPase, beta 3a (atp1b3a)
Dr.4885	NM_131624, AF121796	−4.7	−2.8	−3.8	Solute carrier family 34 (sodium phosphate), member 2a
Dr.6751	AW279637/AL920328, AW280560	−23.5	−4.4	−6.5*	Hemoglobin beta chain
Dr.676	BC050956, BQ618551, BM155115, AA605664	−4.1	−3.7	−8.5	ADP, ATP carrier protein (ADP/ATP translocase 2)
Dr.7076	BI671077, BM186232, BG307552	−2.9	−1.6*	−2.0*	GABA transporter 1
Cytoskeleton structure and dynamics					
Dr.1361	AI330623, BQ264234, BI429216	−2.4	−3.3	−1.5**	Keratin
Dr.2377	AI397158, BQ092519, BE200731, BI884074	−2.8	−2.5	−2.9	Keratin
Dr.25556	BC044144, BE017676, BE200792, AI397347	−2.6	−2.2	−4.3	Keratin
Dr.5531	BE200701	−20.2	−5.5	−10.1	Keratin
Dr.6348	BC047186, AL914133, BF717882, AW116611	−2.6	−1.7	−3.8	Villin 1
DNA/RNA synthesis, processing or degradation					
Dr.11261	BG306387, BM183349	−2.8	−2.4*	−2.0**	Ataxin 2 RNA-binding protein 1
Dr.12233	AW115757, CA472411, BM095899/BI882143, BI887326, AA658595	−32.3	−14.7/−2.3	−30.7/−2.0	Polyadenylate-binding protein 1
Extracellular matrix proteins					
Dr.20126	BM154321, AW171151	−3.9	−2.0	−2.2	Lens gamma-S crystallin
Immune response					
Dr.8238	NM_131570, AF227738	−1.8	−3.3	−3.2	Mannose binding-like lectin (mbl)
Membrane function					
Dr.2936	NM_131771, AF359427	−3.0	−3.4	−4.1	Claudin 10 (cldn10)
Dr.3609	AF428098	−1.8	−2.2	−3.0**	Cadherin-17 (cdh17)
Dr.4188	BI476412, BI325684, BI982373, BI983061	−2.0	−2.3	−2.0	Reticulon 4

Table 3 *(Continued)*

UniGene	GenBank accession numbers	Fold difference			
		Affymetrix	MWG	Sigma	Description
Metabolism					
Dr.10215	AI878786, BM037121, CA975699, AW154364	−2.1	−2.2	−2.3	Phosphoglucomutase
Dr.11729	NM_152954, AF248042	−2.3	−4.5	−4.0	Cytochrome P450 cyp2ja (arachidonic acid epoxygenase)
Dr.11838	NM_131056, AF036326	−3.0	−4.3	−5.0	Preproinsulin (ins)
Dr.12383	BC049331, NM_131798, AJ290390	−3.6	−3.0	−1.9*	Beta-carotene 15,15-dioxygenase (bcdo)
Dr.12454	BI981470, BI534339	−2.6	−2.9	−5.1	GTP cyclohydrolase I feedback regulatory protein
Dr.12949	CA975553, BF938699, BI671189	−17.0	−1.6**	−7.3	Iodothyronine deiodinase type II
Dr.14283	BM316829, BG308877	−2.2	−3.2	−3.4	Adenylate kinase
Dr.14583	AW019656, BI533462, BM072329	−1.8	−2.5*	−2.8*	Amiloride-sensitive amine oxidase
Dr.15151	AI477657, BI889465, BM184016	−2.1	−3.5	−2.6	Esterase D
Dr.1566	NM_131849, AF399909	−2.0	−2.3	−3.3	Alcohol dehydrogenase (ADH3)
Dr.1580	BM071225, AI353103, AI617729	−3.1	−4.8	−5.5	Fructose-bisphosphate aldolase A
Dr.16130	CD014898, AL720129, BM005075	−2.0	−3.1	−4.1	Alcohol dehydrogenase 8b (ADH8b)
Dr.16410	BI474948, CA473262, BF717714	−2.0	−2.6	−2.5	Serine-pyruvate aminotransferase (SPT)
Dr.16655	BC049037, BI980015, BM096422	−2.7	−2.3	−3.6	Glutathione S-transferase
Dr.18834	AW019321, BF717366	−2.9	−4.1	−5.2	Uricase (urate oxidase)
Dr.20080	CA975638, BG303549, AL922875, BG303549	−3.4	−2.6	−2.2	Carbonic anhydrase II
Dr.2045	BI897489, BI705664, BI980068	−2.8	−1.6	−2.2	3-Hydroxyacyl-CoA dehydrogenase precursor
Dr.20966	AF295407	−3.2	−13.3	−12.9	Alcohol dehydrogenase (adh8a)
Dr.23559	AI353354, BE55764, BE202218	−2.2	−4.8	−4.8	Glutathione S-transferase
Dr.23788	NM_131734, AF285098	−1.6	−2.8*	−4.1	Glutathione S-transferase pi
Dr.3275	BG306810, AL909617, BI473436, BG306810	−1.9	−2.1	−2.3	Glycerol-3-phosphate dehydrogenase
Dr.3529	NM_152966, AF189238	−3.5	−2.9	−3.4	Homogentisate 1,2-dioxygenase (hgd)
Dr.3576	AW019349, BF717500	−1.8	−2.5**	−2.3*	Acyl-CoA oxidase
Dr.3647	BM860614, AI477544, AL912051, AI497455	−2.6	−1.7	−2.0	Short chain dehydrogenase
Dr.4111	BC053267, AI437055, AI384201, BG985468	−2.0	−2.7	−2.8	Fructose 1,6-bisphosphatase (fbp)
Dr.4829	AW116228, AL910154, BM082322, BE605341, AW154529, BI891955	−2.7	−2.7	−2.6	5-Aminolevulinate synthase
Dr.6285	AW019758, BI534014	−2.6	−2.2	−3.4	Glycine cleavage system H precursor homolog
Dr.7088	BM024231, BM024714, BM102268	−2.1	−1.9	−2.5	Short chain dehydrogenase
Dr.7557	AW019722, AI546082, AW154722	−2.4	−2.0	−1.8	Ubiquinol-cytochrome *c* reductase core protein ii
Dr.9841	BG306392, BI846916	−2.4	−2.0**	−2.2	cGMP phosphodiesterase
Dr.9860	BC050508, BG738333, BG304707, BE015775, AW826477	−3.3	−2.8	−3.9	Malate dehydrogenase 1b (mdh1b)
Dr.988	AW154697, AW116214	−1.9	−2.2	−2.2	*trans*-Enoyl-coenzyme A isomerase
Muscle-specific genes					
Dr.11483	BC047189, AW133962, BI473729, BG303481	−1.8	−2.3	−4.7*	LIM domain binding 3 like (ldb3l, muscle)
Dr.10694	AF116824	−1.7	−3.0	−3.5	Alpha-cardiac actin
Dr.11252	BC047826, AW116371, BI705975	−5.8	−5.6	−8.3	Creatine kinase

Table 3 *(Continued)*

UniGene	GenBank accession numbers	Fold difference			
		Affymetrix	MWG	Sigma	Description
Dr.1448	AL717344, CA469647, AW281681	−3.6	−4.9	−8.4	Fast skeletal myosin light chain 1A
Dr.16051	BQ615200, BM279699, BM154358	−2.4	−2.5	−2.6*	LIM protein enigma (muscle)
Dr.1699	AI667249, AL908938, AL918509, AW343162	−2.0	−2.6	−3.8	Pyruvate kinase, muscle isoform
Dr.20277	CD014993, AI497570, AW184173, BM024218	−2.0	−3.3	−2.5	Alpha-muscle actin
Dr.20815	BQ783659, NM_131105, BM778643, AF180892, BM154199	−2.6	−4.2	−2.1	Alpha-tropomyosin (tpma)
Dr.21800	AI883923, BM403769, AI794621	−5.0	−2.5	−6.6	Fast-type myosin-binding protein C (MYBP-C)
Dr.23405	BM403462/AI667198, AL914054, AW453743, BG304056, AW454294, BM095209	−26.9	−6.0	−5.2	Myosin heavy chain
Dr.25678	BE201567, BE017652, BM102567, BM811738	−2.7	−2.1	−2.5	Enolase 3 (beta, muscle) (eno3)
Dr.26517	BC053127, AI877831, AI353935, AI353357	−4.1	−4.5	−6.6	Phosphoglycerate mutase 2, muscle form (pgam2)
Dr.28598	CB353610, BQ783571, BM534773, BM155941	−3.6	−1.6*	−2.4	Fast-muscle troponin-i
Dr.373	NM_131196, AF165216	−3.0	−5.8	−7.3	Sk-tropomodulin (Sk-Tmod)
Dr.5224	AW116068, AW128097	−2.4	−1.9	−2.6	Nebulin, sketal muscle
Dr.5549	AF434191, BM156445, AI722973	−2.3	−4.1*	−4.5	Myosin light chain 1 (mlc1)
Dr.636	AW420360, CA473980, BM154034	−2.6	−1.8	−2.3	Myoglobin
Dr.771	AW232091, BI981602, BM103326, AI964264	−2.3	−3.3	−4.5	Creatine kinase, mitochondrial 1 (ckmt1)
Dr.815	NM_131591, AF180887	−3.7	−4.6	−2.9	Skeletal muscle α1 actin (acta1)
Dr.8472	NM_131563, AF180890	−1.6	−3.9	−6.1	Fast skeletal muscle troponin C (tnnc)
Protein synthesis, processing or degradation					
Dr.15327	BM154197, AL917546	−3.0	−2.0**	−6.7*	Elastase I precursor
Dr.13954	CB353596/BI843594, AW595636, BI707054	−37.1/−4.1	−2.7	−3.8	Ribosomal protein L10
Dr.2503	BI880045, CA470963	−1.9	−2.2	−1.9	Calpain, small subunit 1 (capns1)
Dr.4244	AI544815, BF717433	−2.0	−3.6**	−7.4	Carboxypeptidase A1 precursor
Signal perception and transduction					
Dr.14079	BC051156, AL910778, BI710415	−1.8	−1.9	−2.4	14-3-3 Zeta phosphopeptide
Dr.1461	NM_131039, U10403	−2.3	−2.3	−3.8	es1 protein (es1)
Dr.17450	BM956780, BM103565, BM279608, BM103327	−2.1	−2.8	−4.0	Heat shock protein cognate 70 (hsc70)
Dr.26481	CD605019, CA472221, BG727198	−2.5	−2.1**	−2.5	Epidermal growth factor receptor kinase substrate EPS8
Dr.29442	AL721121, BI892249, AI793554	−2.8	−1.8**	−11.7	Cellular retinoic acid binding protein 2 (crabp2)
Dr.8149	NM_131458, AF198033	−2.6	−1.6*	−3.3	Insulin-like growth factor binding protein 2 (igfbp2)
Dr.9881	AW826568, NM_131869, BI671049	−2.4	−2.3	−3.2	Cone transducin alpha subunit (gnat2)
Dr.9922	BI879650, BG308062, BI846866	−2.7	−2.0	−2.3	Cone transducin beta chain 3
Transcription factors					
Dr.267	NM_131312, U66569	−4.5	−1.7**	−1.9*	Myocyte enhancer factor 2c (mef2c)
Other					
Dr.25277	AW232749, BQ093928, AW233227	−3.2	−3.2	−4.2	Anterior gradient 2 homolog
Dr.8097	NM_131192, AF109372	−2.1	−2.0	−2.1	Blue-sensitive opsin (bluops)
Dr.8102	NM_131253, AF109369	−2.6	−1.8	−2.8	Green-sensitive opsin 1 (grops1)

Table 3 *(Continued)*

UniGene	GenBank accession numbers	Fold difference			
		Affymetrix	MWG	Sigma	Description
Dr.8194	BI879950, NM_131319, AF109373	−2.2	−1.9**	−2.2	Ultraviolet-sensitive opsin (uvops)
Unknown					
Dr.23293	BE016153, BE016620	−2.4	−2.3	−2.1	Unknown
Dr.4864	AW019557, AW018657	−2.0	−1.8	−2.8	Unknown
Dr.6913	BC053201, AI544996	−1.9	−2.0	−2.2	Unknown

(A) Genes upregulated by *M. marinum* infection. (B) Genes downregulated by *M. marinum* infection. Criteria for inclusion in the table were: (i) average relative fold difference from the Affymetrix, MWG and Sigma datasets ≥2; (ii) relative fold difference in each separate dataset ≥1.5; (iii) *P*-value for each separate dataset ≤0.05. $0.001 < {}^{*}P \leq 0.01$; $0.01 < {}^{**}P \leq 0.05$, all other *P*-values ≤0.001. Gene descriptions are based on significant homology with human and mouse genes and in some cases have been confirmed by functional studies in zebrafish. The genes were grouped in different categories based on their functions described in the literature or on putative functions inferred from sequence homology to genes with known functions. For the set of downregulated genes, a category of muscle-specific genes was added, which comprises genes with different functions. In a few cases, ESTs from the same UniGene cluster separated into a group of highly induced/repressed genes and a group with lower fold differences. In these cases, the GenBank accession numbers of the two groups and the corresponding values are separated in the table by a forward slash. We note that the *D. rerio* UniGene clusters are not yet completely stable, therefore it is conceivable that the EST groups with inconsistent induction values will eventually be assigned to different UniGene clusters.

Table 4
Immunity-related genes with unchanged expression in response to *M. marinum* infection

Gene	Description	UniGene	GenBank accession numbers		
			Affymetrix	MWG	Sigma
A20	Small inducible cytokine A20 precursor (CCL25)	Dr.9122	BM102177	–	BM102459
ASC1	Apoptosis-associated speck-like protein containing a CARD	Dr.8329	NM_131495	NM_131495	AF231013
CARD15	Caspase recruitment domain-containing protein 15 (NOD2 protein)	Dr.14020	BI864401	–	–
CD83	CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)	Dr.30547	–	–	BM157226
IKKα	Inhibitor of nuclear factor kappa B kinase alpha (CHUK)	Dr.14012	BC051614	BQ261936	BM071802
ECSIT	Evolutionarily conserved signaling intermediate in Toll pathway	Dr.2345, Dr.32817, Dr.36395	BM316149	AL927530	BF158409
IFN	Interferon	Dr.26701	AY135716	–	–
IKAP	I kappa B kinase complex-associated protein	Dr.9310	BI879503	BI879503	–
IKKγ	Inhibitor of nuclear factor kappa B kinase gamma (NEMO)	Dr.6051	BM026080	–	AW076974
IL1RAP	Interleukin-1 receptor accessory protein-like 1	Dr.29232	–	AW134384	AW116693
p38	Mitogen-activated protein kinase 14a	Dr.7930	NM_131722	NM_131722	AB030897
MyD88[a]	Myeloid differentiation primary response gene 88	Dr.30199	–	AY389463	–
NLK	NEMO-like kinase	Dr.30386	–	BG304985	–
RelA	Nuclear factor kappa B RelA (p65)	Dr.31000	–	BI887255	–
RelB	Nuclear factor kappa B RelB	Dr.9524	AW420676	–	–
RIP-4a[b]	Protein kinase C-associated kinase (PKK)/receptor interacting protein 4	Dr.18839	BC045432	–	BM101597
ScarB1	Scavenger receptor class B, member 1 (CD36 antigen)	Dr.6652	BC044516, BI983877	–	BE202194
TAB1	TAK1-binding protein 1	Dr.16754	CA469739, BI981448	AL922594	BI981448
TBK-1	TANK-binding kinase 1 (NFκB-activating kinase)	Dr.9951	–	BE200581	BE016392
TIGGIR	Interleukin-1 receptor accessory protein-like 2	Dr.13541	BI865738	–	BI865738
TLR21	Toll-like receptor 21	Dr.18923	BM185313	–	–
TNFRa	Tumor necrosis factor receptor superfamily, member a	Dr.10712	CD777695, NM_131840	NM_131840	AF250042
TRAF6	TNF receptor-associated factor 6	Dr.15109	BM182395	BM182735	BM182395

Genes included in the table showed less than 1.5-fold difference between control and infected fish or differences were not significant ($P > 0.05$).

[a] Custom oligonucleotides for MyD88 were added to the MWG Oligo Set in our microarray experiments.

[b] Another RIP family member (RIP-2, Dr.28180, BG728701) was 2.1-fold ($P = 0.001$) induced in the Sigma microarray analysis.

Table 5
Immunity-related genes not present on the Affymetrix, MWG and Sigma microarrays

Gene	Description	UniGene cluster or genomic contig
CARD4	Caspase recruitment domain-containing protein 4 (NOD1 protein)	Zv4_NA18690
CD27	CD27 antigen (tumor necrosis factor receptor superfamily, member 7)	Zv4_scaffold1385
CD28	T-cell antigen CD28 and CD152 (CTLA-4) homolog	Zv4_scaffold795.1
CD36	Cell adhesion receptor CD36	Dr.31525
CD80	CD80 antigen (B-lymphocyte activation antigen B7-1)	Zv4_NA14820
CD86	Putative CD86 antigen (B-lymphocyte activation antigen B7-2-like)	Zv4_scaffold1252/Zv4_scaffold16
cREL	Nuclear factor kappa B c-Rel	Dr.30999
I kappa B	Inhibitor of nuclear factor kappa B	Dr.26671
IKKβ	Inhibitor of nuclear factor kappa B kinase beta	Zv4: AL954172.9
IL-10	Interleukin-10	Zv4: BX321912.5
IL-12α	Interleukin-12α (p35)	Dr.32161
IL-12β	Interleukin-12β (p40)	Dr.32162
IL-18R	Interleukin-18 receptor	Dr.30198
IL-1β	Interleukin-1β	Dr.30443
IL-1R	Interleukin-1 receptor	Dr.30441
IL-2	Interleukin-2	Zv4_scaffold555
IL-8	Interleukin-8	Dr.30763
iNOS	Inducible nitric oxide synthase	Dr.30520
IRAK-M	Interleukin-1 receptor-associated kinase M	Zv4_scaffold1136
IRF-3	Interferon regulatory factor 3	Zv4_scaffold1903
MAL/TIRAP	MyD88-adaptor like protein	Dr.30201
MD1	MD1 protein	Zv4_NA11849
NFκB1	Nuclear factor kappa B 1 (p105)	ZV4_NA8225.1
RIP-1	Receptor-interacting serine/threonine kinase1/receptor interacting protein 1	Zv4: AL935300.10
RP105	Lymphocyte antigen 64, RP105	Zv4_scaffold1273
SARM	Sterile alpha and heat-Armadillo motifs	Dr.30197
Sigirr	Single immunoglobulin domain-containing IL-1R-related protein	Zv4_scaffold2031
SLAM	Signaling lymphocyte activation molecule	Zv3_NA10998
ST2	Suppressor of tumorigenicity 2	Zv4_scaffold1202
TAB2	TAK1-binding protein 2	Zv3_NA18314/ctg22363
TLRs	Toll-like receptors (TLR1, TLR2, TLR3, TLR4a, TLR4b, TLR5a, TLR5b, TLR7, TLR8a, TLR8b, TLR9, TLR18, TLR19, TLR20a, TLR20f, TLR22)	Dr.30180-84, Dr.30186-96
TNF	Tumor necrosis factor	Dr.30430
TRIF	TIR-domain adaptor inducing IFN-β	Dr.30200

Oligonucleotide probes corresponding to ESTs from the indicated UniGene clusters are not included in the Affymetrix, MWG and Sigma microarrays. Genes without UniGene codes were identified in the zebrafish whole genome shotgun assembly (version Zv3 or Zv4) of the Sanger Institute and the genomic contig number is indicated in the table. Oligonucleotide probes corresponding to these genes could not be found on the microarrays. A number of other immune genes have not yet been identified in the zebrafish genome shotgun sequence, including CD4, CD8, CD14, CD19, MD2, IKIP, IL-6 and IL-18.

genes. Therefore, we checked the presence of other immunity-related genes on the different microarrays. Table 4 lists genes that were present on one or more of the microarrays but showed no significant expression differences. Previously we demonstrated by RT-PCR analysis that expression of several Toll-like receptor (TLR) genes was increased in response to *M. marinum* infection (Meijer et al., 2004). However, no TLRs were present on the microarrays, except TLR21, which was not induced in agreement with our reported RT-PCR results. Different signaling intermediates of the TLR pathway were also not induced (MyD88, ECSIT, TRAF6, TAB1) or not present (e.g. MAL, TRIF) on the microarrays, with exception of the Toll interacting protein, TOLLIP, and the interleukin-1 receptor associated kinase 4, IRAK4, which were both induced (TOLLIP: BC046009/BI979095; Affymetrix: 1.9-fold ($P < 1.00E-5$); Sigma: 2-fold ($P < 0.001$); IRAK4: BC045381/BQ783846; Affymetrix: 2.4-fold ($P < 1.00E-10$); MWG: 2.1-fold ($P < 1.00E-10$)). The response of TLR signaling components to mycobacterium is of special interest because it has recently been shown that mycobacterial lipoprotein inhibits IFN-γ signaling in a TLR2- and MyD88-dependent fashion, possibly contributing to persistence of mycobacterial infection (Fortune et al., 2004). Four members of the nuclear factor kappa B family were not induced (RelA, RelB) or not present (cRel, NFκB1) on the microarrays, but NFκB2 (BI881888) showed 1.85-fold induction ($P < 1.00E-5$) in the Affymetrix analysis. We checked the zebrafish genome shotgun sequence for the presence of other immunity-related genes that have been characterized in human and mouse. The zebrafish counterparts of many such genes could be identified, but no corresponding ESTs could be found in the microarrays (Table 5).

3.4. Quantitative RT-PCR analysis

To verify the data obtained by microarray analysis, quantitative RT-PCR (qPCR) was performed on six genes (Fig. 3). We picked two upregulated genes and one downregulated

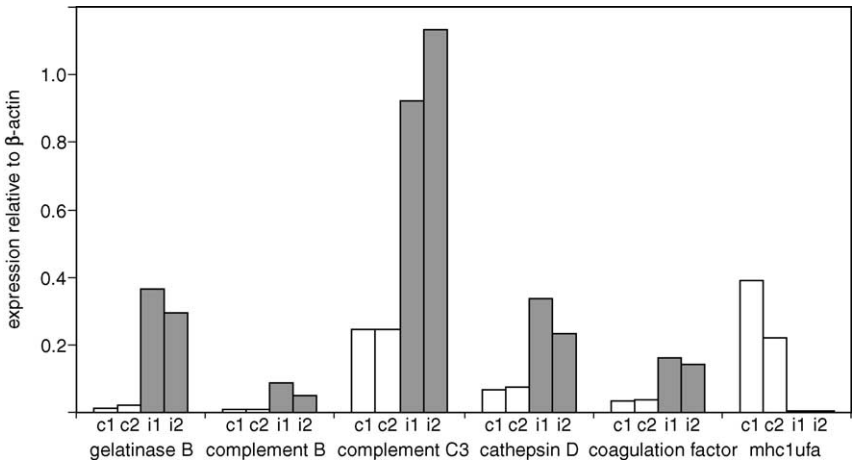

Fig. 3. Confirmation of microarray results by qPCR analysis.Bars represent the fold difference in expression level compared to the β-actin housekeeping gene. RNA samples were from two control fish (c1 and c2) and two *M. marinum*-infected fish (i1 and i2).

gene from the reference set that showed differential expression in all microarray types (Table 1). In the qPCR assay, gelatinase B (matrix metalloproteinase 9; AW174507) and cathepsin D (NM_131710) were, respectively, 20.4- and 4.0-fold higher expressed in the infected fish compared to the control fish. This correlated with the microarray results from Affymetrix, MWG and Sigma, respectively, in which gelatinase B showed 13.3-, 10.9- and 12.8-fold induction and cathepsin D showed 2.9-, 3.5- and 1.6-fold induction. The qPCR analysis further showed that expression of the major histocompatibility class 1 UFA gene (mhc1ufa; AF137534) was decreased to undetectable levels in the infected fish, in agreement with the microarray data that also indicated strong downregulation (100-, 36.3- and 20.3-fold in Affymetrix, MWG and Sigma, respectively). We also picked three genes for qPCR analysis that were only present on one or two of the microarray types. Microarray analysis detected that these genes, coding for complement factor B (AI601749), complement factor C3 (BM095286/AW116558) and a coagulation factor homolog (AL590146_3), were upregulated in the infected fish compared to the controls (5.4-fold (MWG), 4.6/3.0-fold (MWG/Sigma) and 6.6-fold (MWG), respectively). The results of qPCR analysis confirmed the increased expression of these genes in the infected fish (8.0-, 4.2- and 4.3-fold, respectively).

4. Discussion

To increase knowledge of host responses to mycobacterial infections we have analyzed the transcriptome profile of zebrafish after chronic exposure to *M. marinum* infection. The combined use of microarrays from three different platforms (Affymetrix, MWG and Sigma) has resulted in the identification of a set of 159 genes whose differential expression is detected irrespective of the type of microarray chosen. This set will be very useful as a multiplatform reference for further transcriptome profiling studies in the *M. marinum*–zebrafish infection model. In the datasets from each individual platform, we found a largely similar distribution of mycobacterium-induced genes over different functional categories, leading to the same general conclusions. In particular, the proteins encoded by all three sets of induced genes included a large group of lysosomal proton pumps, different components of the complement system, specific B- and T-cell proteins, enzymes of the macrophage defense mechanisms, and homologs of many receptors and signal transduction proteins with known immunity-related functions. The added value of analyzing different microarrays is that more genes can be analyzed, since not all relevant immunity-related genes are represented in all platforms. For future larger scale screens it would be useful to design a zebrafish microarray that includes all these genes as well as a number of other immune genes that are currently not represented by any of the existing platforms (Table 5). In this respect, spotted oligonucleotide microarrays offer the highest flexibility for addition of custom oligonucleotides. Furthermore, spotted microarrays that have been validated by comparative analyses provide a cost effective alternative for Affymetrix GeneChips as we have shown here for the MWG and Sigma zebrafish oligonucleotide sets.

While other studies have used in vitro systems, this is the first time that a host transcriptome response to mycobacterium infection is studied at the organismal level. In the future, it will be of great interest to compare the results with data from the mouse tuberculosis model and from human tuberculosis patients. Several recent studies have used human blood monocytes or cell cultures of macrophages and dendritic cells to determine the host transcriptional response to *M. tuberculosis* infection (see Section 1). We observe various similarities between the reported transcriptome profiles and that of the *M. marinum*-infected zebrafish. For example, we detected strong inductions of different matrix metalloproteinases and some of their inhibitors in zebrafish, which was also observed in at least three reports on human in vitro systems (Ragno et al., 2001; Chaussabel et al., 2003; Wang et al., 2003).

Furthermore, a matrix metalloproteinase was also found to be induced in a transcriptome analysis of blood monocytes from *Mycobacterium avium*-infected cattle (Coussens et al., 2003). Matrix metalloproteinases have been thought to be involved in remodeling of the extracellular matrix. However, recent findings indicate that the matrix metalloproteinases have much broader functions in the regulation of inflammation and immunity, affecting the activity of cytokines, chemokines and other proteins (Parks et al., 2004). Other examples of similarities between the transcriptome profiles of *M. marinum*-infected zebrafish and *M. tuberculosis*-infected cells are the induction of osteopontin (Ragno et al., 2001; Wang et al., 2003), a secreted cytokine with cell adhesive and chemoattractive functions, the induction of Janus kinase 1, which regulates cytokine signal transduction (Chaussabel et al., 2003), and the induction of components of the phagocyte oxidase system (e.g. p47-phox (Wang et al., 2003)) involved in superoxide generation. Furthermore, the induction of different apoptosis-related proteins detected in zebrafish (e.g. caspase a, c-IAP) parallels observations in human macrophages and alveolar epithelial cells (Danelishvili et al., 2003).

In zebrafish, different molecular markers for macrophages and granulocytes have been described. These include among others Spi1 (PU.1), a transcriptional regulator of commitment to the myeloid lineage, L-plastin, an actin-bundling protein with probable functions in adhesion and activation, fms, the receptor for macrophage colony-stimulating factor, the C-type lysozyme and mpx, a peroxidase specific for heterophil granulocytes (Herbomel et al., 1999; Crowhurst et al., 2002; Liu and Wen, 2002). Our microarray analyses detected increased expression of these five markers in the *M. marinum*-infected fish. In addition to Spi1, three members of the c/ebp transcription factor family are also known as markers of myeloid precursors in zebrafish (Lyons et al., 2001a,b). Two of these (the zebrafish orthologs of mammalian c/ebpα and c/ebpβ) were induced in the infected fish, while the third (the fish-specific c/ebp1 gene) showed unchanged expression. Increased expression was also detected of homologs of human myeloid cell markers that have not yet been studied in zebrafish, for example the tartrate-resistant acid phosphatase 5. Such genes can be used to expand the toolbox for analysis of myeloid development in zebrafish. This would also support zebrafish mutagenesis screens, which are seen as a powerful means to discover new genes involved in myelopoiesis.

The infected fish showed upregulated expression of many genes coding for proteins with functions in cytoskeleton dynamics, which are likely to be involved in the activation programs of different cell types of the immune system. One of these genes, coronin (TACO), has received attention as a candidate host factor that might play a role in the mechanism by which mycobacteria manage to survive intracellularly. It was suggested that active retention of coronin on the phagosomal membrane by mycobacteria prevents fusion with the lysosomal compartment (Ferrari et al., 1999). However, this hypothesis has been questioned by other studies, which have implicated coronin only in mycobacterial phagocytosis but not in phagosome maturation (Schuller et al., 2001; Solomon et al., 2003). The infected fish showed also increased expression of genes encoding other proteins with roles in phagosome maturation, including a SNARE syntaxin, the small GTP-binding protein, Rab7, and the lysosome-associated membrane glycoprotein 1 (LAMP1). It is doubtful if the transcriptional upregulation of these genes is effective against pathogenic mycobacteria, which are thought to have evolved the ability to interfere with the membrane tethering and fusion machinery involved in the maturation of phagosomes (Flynn and Chan, 2003).

The set of genes that were downregulated in the infected fish included a wide range of muscle-specific genes, which may simply reflect the general disease state of the animals, in which mobilization of reserves is needed to combat infection. More specific for the mycobacterial disease might be the downregulation of MHC class genes. Mycobacteria may evade immune surveillance by inhibiting antigen presentation by MHC class II proteins or by the alternate MHC-I processing machinery (Flynn and Chan, 2003). It has been shown that prolonged exposure of macrophages to *M. tuberculosis* inhibits antigen processing by MHC-I and MHC-II and furthermore inhibits MHC-II expression at the transcriptional level (Tobian et al., 2003). In our study, we detected transcriptional repression of not only of MHC-II, but also of MHC-I, which has not been previously reported as a possible mechanism used by mycobacteria to establish chronic infection.

It is likely that the set of differentially expressed genes includes a number of genes that represent more general markers of disease rather than markers of a directed immune response. However, there also several examples that suggest a response specifically related to the infection process. For instance, the group of upregulated metabolic genes includes cholesterol 24-hydroxylase, which enzyme is required for metabolism of cholesterol, which was proposed to play an essential role in the entry of mycobacteria into and survival within macrophages (Gatfield and Pieters, 2000). Another example indicative of specificity is that only a relatively small group of transcription factor genes was induced. This small group included myeloid-specific transcription factors described in zebrafish (Spi1, c/ebpα, c/ebpβ) and homologs of transcriptional regulators of the mammalian immune response (e.g. Ikaros, LITAF, BCL5, GILZ). In addition, we observed that within different gene families, only certain members were specifically induced. For example, several matrix metalloproteinase genes were induced, but many other members of the family showed unchanged expression. Another good example concerns a chitotriosidase (gp39c, BC046004/AW019171), belonging to the chitinase family, which was strongly induced in the *M. marinum*-infected zebrafish (Affymetrix: 9.1-fold ($P < 1.00E{-}10$); Sigma: 100-fold ($P = 0.008$)). This observation is interesting because a human chitinase has recently been associated with inflammatory responses (Zhu et al., 2004). Furthermore, elevated expression of a human chitotriosidase that is secreted by ac-

tivated macrophages, is correlated with atherosclerosis and Gaucher disease, a lysosomal storage disorder (Boot et al., 1999). Several other zebrafish chitotriosidase-related genes (gp39a, gp39b, gp39d), with chromosomal location close to the inducible gp39c gene, were not induced by *M. marinum* infection. This could indicate a specific role for this particular member of the chitinase family in inflammatory responses.

Several recent studies have exploited the *M. marinum*–zebrafish interaction as a new surrogate model to advance understanding of the pathogenesis of human tuberculosis. The transcriptome response of zebrafish to *M. marinum* infection that we report here provides further validation for this disease model by demonstrating the differential expression of many zebrafish homologs of human genes that are involved in immune responses in general or that are associated with the response to *M. tuberculosis* infection in particular. In future work we will compare the zebrafish transcriptome profile of chronic exposure to *M. marinum* with that of the early response to *M. marinum* infection and make comparisons between responses to different *M. marinum* strains and responses to other pathogens. These studies may reveal transcriptional responses specific for mycobacterial diseases and thereby provide valuable leads for further investigation of the process of mycobacterial pathogenesis.

Acknowledgements

This work was supported by a European Commission 6th Framework Programme grant (contract LSHG-CT-2003-503496, ZF-MODELS). In addition, the lab of Robert Geisler was supported by the German Human Genome Project (DHGP grant 01 KW 9919). Fons J. Verbeek is partially supported by the Netherlands Organization for Scientific Research (NWO-CW, Molecular Bioinformatics Programme). We thank André Wijfjes and Wilbert van Workum (ServiceXS, Leiden, The Netherlands) for their expert advice on microarray analysis, Wilbert Bitter (VU Medical Centre, Amsterdam, The Netherlands) for critically reading the manuscript and Matthias Bauer (Institute of Toxicology and Genetics, Forschungszentrum Karlsruhe, Germany) for advice on spotting the Sigma OligoLibrary.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molimm.2004.11.014.

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