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Transcriptome profiling of zebrafish infected with *Streptococcus suis*

Zongfu Wu, Wei Zhang, Yan Lu, Chengping Lu*

Key Lab Animal Disease Diagnostic and Immunology, Ministry of Agriculture, Nanjing Agricultural University, Weigang 1, Nanjing 210095, Jiangsu, China

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ABSTRACT

Streptococcus suis is an important pathogen in swine, and it also represents an emerging zoonotic agent. Zebrafish as a model for the evaluation of virulence of *S. suis* has been demonstrated before. Here, an Affymetrix Zebrafish GeneChip was used to identify alterations in gene expression of zebrafish injected with *S. suis* serotype 2 strain HA9801. The results showed that 189 genes were differentially expressed, of which 125 genes were upregulated and 64 genes were downregulated. Gene Ontology category and KEGG pathway were analyzed for differentially expressed genes. Upregulated genes were involved in response to bacterium, immune response, inflammatory response, complement activation, defense response. Three genes (encoding serum amyloid protein A, matrix metalloproteinase 9 and apoptosis-related cysteine protease) and genes involved in the regulation of IL-6 biosynthetic process, which have previously been implicated in the response to *S. suis* infection in other organisms, were also upregulated. Downregulated genes played roles in glycolysis, carbohydrate metabolic process, amino acids metabolism, behavior and muscle. The reliability of the data obtained from the microarray was verified by performing quantitative real-time PCR on 12 representative genes. The data may provide further validation of this model, which will contribute to understanding of *S. suis* pathogenic mechanisms.

1. Introduction

Streptococcus suis is an important pathogen associated with a range of diseases in pigs, including meningitis, pneumonia, septicemia, and arthritis [1,2]. Human infection with *S. suis* has become a serious zoonosis and has been reported in different Asian and European countries, as well as in New Zealand, Australia, Argentina, and Canada [3,4]. There are presently 35 serotypes of *S. suis* (serotypes 1–34 and serotype 1/2) recognized on the basis of capsular antigens [2]. Most studies on the pathogenesis of *S. suis* are based on *S. suis* serotype 2 (SS2). Animal models are essential to achieve a better understanding of pathogenesis of *S. suis*. Mice were used as model for evaluation of virulence of *S. suis* [5,6]. Recently, a hematogenous model of infection in CD1 mice was developed by Dominguez-Punaro et al. [7]. In addition, their further research demonstrated that A/J mice were significantly more susceptible to *S. suis* infection than B6 mice, especially during the acute septic phase of infection [8]. These experimental models may be useful for studying the pathogenic mechanisms of *S. suis*. However, the concept of virulence may differ depending on the experimental model that is used, and this has hampered study of the pathogenesis of this bacterial species. Different research groups use different animal species, pigs of different age and immunological status, or different routes of infection [9,10]. This variation in the experimental systems used may result in important discrepancies regarding the virulence of even the same strain [10–12].

Its well-developed adaptive and innate cellular immune systems make the zebrafish an ideal model for the study of infectious diseases [13–15]. Although the use of zebrafish as an infection model is a relatively new development, several different pathogens have already been studied using this model [13,14,16]. Infection of adult zebrafish has been successfully demonstrated with pathogens such as *Streptococcus* spp. [15], *Mycobacterium marinum* [17], *Francisella* sp. [18], *Aeromonas salmonicida* [19], and *Listeria monocytogenes* [20]. Zebrafish embryos have been demonstrated as infection model for *Staphylococcus aureus* [19,21], *Salmonella typhimurium* [22], and *M. marinum* [23]. Zebrafish as a model for the evaluation of virulence of *S. suis* has been demonstrated by our group [24,25], which has been cited by the World Organisation for Animal Health (OIE) [26]. SS2 strain HA9801, originally isolated by our group, is considered to be a virulent strain [25,27–29]. SS2 strain T15, obtained from Dr. H.E. Smith of the DLO-Institute for Animal Science and Health in the Netherlands, is an avirulent strain

Abbreviations: SS2, *Streptococcus suis* serotype 2; SS9, *Streptococcus suis* serotype 9; LD_{50}, 50% lethal dose; cfu, colony forming units; PCPEC, porcine choroid plexus epithelial cells; MMP-9, matrix metalloproteinase 9; SAA, serum amyloid A; BBB, blood brain barrier; CSF, cerebrospinal fluid.

* Corresponding author. Tel./fax: +86 25 84396517.
E-mail address: lucp@njau.edu.cn (C. Lu).

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and serves as a reference avirulent strain standard [28,30,31]. The 50% lethal dose (LD_{50}) value of strain HA9801 was 1.85×10^4 cfu/fish, whereas zebrafish injected with strain T15 exhibited no mortalities [24]. *S. suis* serotype 9 (SS9) strain GZ0565 was isolated from a diseased pig with meningitis, and SS9 strain SH040917 was isolated from the tonsils of a healthy pig [25]. We previously evaluated the virulence of these two SS9 strains in zebrafish—the LD_{50} value of strain GZ0565 was 3.8×10^5 cfu/fish, whereas zebrafish injected with strain SH040917 exhibited no mortalities [25]. Strains GZ0565 and SH040917 were proven to be pathogenic and nonpathogenic, respectively, in an experimental pig model [32]. These studies have demonstrated that the zebrafish is an ideal model for evaluating the virulence of *S. suis*.

Furthermore, compared with pigs and mice, the zebrafish offers many advantages as a model for the evaluation of virulence of *S. suis*, including low cost, easy maintenance, requirement of minimal laboratory space, and easy handling. The low cost and small size of zebrafish allow the testing of individual isolates from a large pool of potential mutants, which may contribute to an understanding of *S. suis* pathogenic mechanisms. Therefore, in this study, microarray analysis was used to identify alterations in the gene expression of *S. suis*-infected zebrafish, with the aim of providing further validation of this model.

2. Results

2.1. S. suis infection in zebrafish

The mortality for zebrafish injected with a dose of 3×10^6 cfu/fish between 24 h and 96 h was 80%. Mortality was monitored until 7 days post-infection. The moribund fish exhibited reddening of the abdomen (Fig. 1), became lethargic, and swam near the surface of the water. Control fish showed no mortalities or signs of disease over a period of 7 days. On the basis of these observations, experimental fish were injected with 3×10^6 cfu/fish. At 20 h post-infection, three infected fish and three control fish were selected.

2.2. Microarray analysis and Gene Ontology category

Triplicate microarray results revealed that a total of 189 genes exhibited a $\geq$2-fold or $\leq$0.5-fold change in induction or suppression, of which 125 genes were upregulated and 64 genes were downregulated (Table 1).

These differentially expressed genes were classified into different functional categories according to Gene Ontology (GO) project for biological process. The main GO categories for upregulated genes were response to bacterium, immune response, antigen processing and presentation, inflammatory response, macrophage chemotaxis, complement activation, defense response, platelet activation, blood coagulation, protein polymerization, proteolysis, ion transport, CD8-positive alpha-beta T cell differentiation, positive regulation of interleukin-12 biosynthetic process, regulation of interleukin-6 biosynthetic process, and positive regulation of apoptosis (Fig. 2A). The primary GO categories for downregulated genes were carbohydrate metabolic process, behavior, muscle thin filament assembly, actin–myosin filament sliding, glycolysis, creatine biosynthetic process, proteoglycan metabolic process, L-phenylalanine catabolic process, tyrosine metabolic process, purine base metabolic process, folic acid metabolic process, xylulose metabolic process, bile acid metabolic process, tyrosine catabolic process, phosphate transport, DNA strand elongation, DNA replication proofreading, and DNA synthesis during DNA repair (Fig. 2B). The differentially expressed genes involved in significant GO categories are summarized in Supplementary Table 1.

2.3. Pathway analysis

The KEGG pathway analysis for upregulated genes showed that the genes were involved in complement and coagulation cascades, antigen processing and presentation, leukocyte transendothelial migration, and proteasome (Fig. 3A). The KEGG pathway analysis for downregulated genes also showed that the genes were related to glycolysis and amino acids metabolism, including glycine, serine, threonine, cysteine, methionine, pyruvate, and arginine (Fig. 3B). The differentially expressed genes involved in significant pathway are summarized in Supplementary Table 2.

2.4. Confirmation of microarray results by quantitative real-time PCR

In order to verify the data obtained by microarray analysis, quantitative real-time PCR was performed on 12 genes. We selected 5 upregulated genes and 7 downregulated genes that exhibited differential expression in the microarray. As shown in Fig. 4, the results confirmed the data from the microarray.

3. Discussion

While several studies have used human macrophages cells, porcine choroid plexus epithelial cells (PCPEC), or porcine brain microvascular endothelial cells to determine the host response to *S. suis* infection [33–35], this was the first time that a host transcriptome response to *S. suis* infection has been studied at the organismal level. We observed several similarities between these reports from *S. suis*-infected cells and our transcriptome profile of *S. suis*-infected zebrafish. For example, we detected the induction of matrix metalloproteinase 9 (MMP-9) in *S. suis*-infected zebrafish, which was also observed in studies on human macrophage cells [33]. Jobin et al. have shown that whole cells of *S. suis* are able to upregulate the production of MMP-9 by human macrophage cells, and *S. suis*-mediated MMP-9 production by human macrophages may play a critical role in blood brain barrier (BBB) disruption and tissue destruction [33]. MMP-9 is a metalloproteinase that is active against matrix proteins and is secreted by various cell types. Pathophysiological processes characteristic of bacterial meningitis, such as neutrophil extravasation,

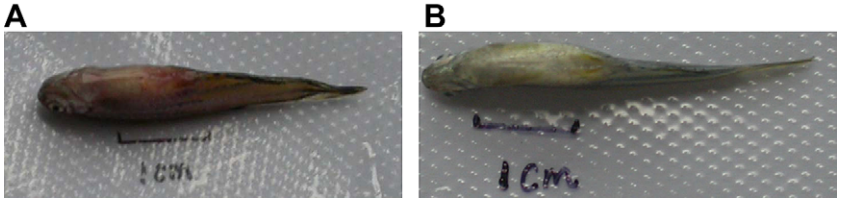

Fig. 1. *S. suis* infection in zebrafish. (A) Gross pathology of zebrafish infected with *S. suis*. The moribund fish showed reddening of the abdomen and became lethargic and swam near the surface of the water. (B) Control fish showed no mortalities or signs of disease over a period of 7 days.