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Microarray analyses of THP-1 cells infected with *Streptococcus suis* serotype 2

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ABSTRACT

Streptococcus suis serotype 2 (*S. suis* 2) is a pathogen responsible for several diseases in both pigs and humans. To gain more insight into the pathogenesis of this organism, an oligonucleotide (oligo)-based microarray was used to investigate gene expression changes in human monocytic cells (THP-1) in response to exposure to *S. suis* 2 strain SC19. A total of 328 differentially expressed genes were identified. These differentially expressed genes belonged to a variety of functional categories, including genes involved in apoptosis, immunity, signal transduction, chemokine production and the ubiquitin–proteasome system. Our findings can be of interest for future research.

1. Introduction

Streptococcus suis is an important swine pathogen that causes meningitis, septicemia, arthritis, bronchopneumonia and endocarditis and leads to significant economic losses worldwide. It is also an important zoonotic agent affecting individuals who come into contact with swine or their by-products (Okwumabua and Chinnapapakkagari, 2005). Human infections with *S. suis* can result in meningitis, septicemia, permanent hearing loss (in 50% of cases), pneumonia, endocarditis, arthritis and toxic shock syndrome.

S. suis is a Gram-positive bacteria for which 33 serotypes have been reported to date, based on discrepancies in their capsular antigens (Gottschalk et al., 1998). *S. suis* 2 is the most frequently isolated serotype of *S. suis* in both swine and humans. In 1998 and 2005, *S. suis* 2 outbreaks in humans were reported in China, affecting hundreds of people and leading to several fatalities. In 2005 outbreak, the two major clinical syndromes were streptococcus meningitis syndrome (SMS) and streptococcus toxic shock syndrome (STSS). STSS is characterized by high fever, generalized petechiae and purpura, multiorgan insufficiency, shock and Disseminated Intravascular Coagulation (DIC) with a high mortality rate (Tang et al., 2006; Yang et al., 2009).

A previous study reported that *S. suis* is able to adhere to different epithelial cells in a time- and dose-dependent fashion (Lalonde et al., 2000), and can induce programmed cell death upon long-term incubation and then enter into the bloodstream. Host–pathogen interactions during *S. suis* infection are complex. To study the molecular mechanisms underlying the host response to pathogenic microorganisms in monocytes, microarrays have been widely used in recent years. The aim of this study was to improve our understanding of the host–pathogen interactions during *S. suis* 2 infection.

2. Materials and methods

2.1. Bacterial strains and growth conditions

S. suis 2 strain SC19 was isolated from diseased pigs in the Sichuan province of China. Bacteria were grown on

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blood agar plates (tryptic soy agar (TSA; DifcoTM, France) containing 10% bovine blood) for 18 h at 37 °C, and inoculated into serum broth (tryptic soy broth (TSB; DifcoTM) containing 10% bovine blood) for 6 h at 37 °C. Bacteria were centrifuged at 12,000 × *g* for 5 min to pellet the bacteria, washing twice with phosphate-buffered saline (PBS), pH 7.4, and then diluted to approximately 1×10^9 colony-forming units (CFU)/mL.

2.2. Cell line and culture conditions

The human monocytic cell line THP-1 (ATCC TIB-202) was grown and maintained in 1640 medium containing 10% (v/v) fetal bovine serum (FBS), 50 mM 2-mercaptoethanol (2-ME) and at 37 °C in a 5% CO_2 atmosphere.

2.3. Exposure of cells to S. suis 2 strain SC19

For the stimulation assays, 48 h-cultures of THP-1 cells were washed twice with PBS and resuspended in 1640 maintenance medium. *S. suis* 2 strain SC19, diluted appropriately in maintenance medium, was added to THP-1 cells at a multiplicity of infection (MOI) of 1:1 (bacteria/cells). Uninfected control cells were incubated with 1640 maintenance media only (mock infected). After a 3-h incubation period, cells were collected by centrifugation at 300 × *g* for 5 min and the supernatant was then discarded.

2.4. RNA extraction, reverse transcription, RNA labeling and cRNA hybridization

After exposure to bacteria, cells were centrifuged in RNase-free tubes treated with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and stored at −80 °C prior to RNA extraction. Total RNA extraction from cells was performed according to the manufacturer's standard instructions (Invitrogen) and then the RNA was then prepared and purified using the RNeasy Mini Kit (QIAGEN China (Shanghai) Co., Ltd.). RNA quality was assessed by microcapillary electrophoresis on an Agilent 2001 Bioanalyser (Agilent Technologies) with RNA 6000 Nanochips. RNA was quantified by spectrophotometry (ND-1000; NanoDrop Technologies).

2.5. Microarray hybridization

RNA was labeled using the recommended protocols supplied by the manufacturer (Affymetrix). Briefly, double-stranded cDNA was synthesized from 6 μg of total RNA using a T7-oligo(dT) primer. Then the cDNA was purified and converted into cRNA via an *in vitro* transcription reaction in the presence of biotinylated nucleotides. Subsequently, the biotinylated cRNA was purified and quantified by spectrophotometric methods. The cRNA yield was adjusted for the carryover of unlabeled total RNA. Twenty micrograms of cRNA were fragmented for 35 min at 94 °C and then hybridized to Affymetrix Human Genome U133 Plus 2.0 GeneChip arrays for 16 h. After hybridization, the GeneChips were washed and stained using a GeneChip Fluidics station 450 (Affymetrix) and were then scanned with an Affymetrix GeneChip scanner. Affymetrix GeneChip Human Genome U133 Plus 2.0 arrays contains over 54,000 probe sets, 47,000 transcripts and variants, including 38,500 well-characterized human genes.

2.6. Microarray data analysis

For microarray data analysis, the Affymetrix expression data were analyzed using the software package Gene Spring, version 7.31 (Agilent). All experiments (including infected and mock-infected cells) were performed in triplicate to allow the assessment of in-group variation. First, the expression data obtained from infected cells were normalized to the median of the uninfected control samples. An initial selection of genes was made based on a two-fold change in expression relative to that of uninfected control cells. Subsequently, an error model based on replicate measurements was defined using the Gene Spring software, and the significance of differential expression was established using analysis of variance and the false-discovery rate method of Benjamini and Hochberg (1995) to correct for multiple testing. Microarray analysis was then performed by the commercial Affymetrix array service (GeneTech Biotechnology Limited Company, Shanghai, China. The data discussed in this study have been deposited in NCBI's Gene Expression Omnibus, accession number is GSE20508.

2.7. Real-time PCR

Real-time quantitative PCR was used to validate selected data from the microarray experiments and to follow the expression of a subset of genes over time. Total RNA was reverse transcribed using the first strand cDNA synthesis kit (ReverTra Ace –a-^{TM}, Toyobo, Japan) according to the manufacturer's instructions. GAPDH was used as endogenous control. The specific primers used for the RT-PCR assays are listed in Table 1. The SYBR Green method was used and all reactions were performed in triplicate. Each reaction tube contained 25 μL 2×SYBR Green real-time PCR Master Mix, 0.5 U mL^{-1} RNAse inhibitor, 0.3 U mL^{-1} ReverTra Ace (Toyobo, QPK-201), 0.4 mM of gene-specific F and R primers and 200 ng of template RNA, made up to a final volume of 50 mL with distilled water. The RT-PCR cycling conditions were as follows: 42 °C for 30 min and 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 °C for 45 s. For relative quantitative RT-PCR, an ABI 7500 real-time PCR system (Applied Biosystems, Warrington, UK) was used. Relative standard curves for the target and endogenous control primer pairs were performed to verify that both PCR efficiencies were comparable and, therefore, the comparative $2^{-\Delta}\Delta$Ct method (Livak and Schmittgen, 2001) was applied.

3. Results

3.1. Gene expression analysis during S. suis 2 infection

To investigate the pathogenesis of *S. suis* 2, the differential gene expression profile of THP-1 cells, a human