THE TRANSCRIPTIONAL RESPONSE TO *Salmonella* INFECTION IN SWINE

Shawn M.D. Bearson*, Jolita J. Uthe1,2, Atabak R. Royaee3, Joan K. Lunney3, Shu-Hong Zhao2, Jae Woo Kim2, Chris K. Tuggle2

*USDA, ARS National Animal Disease Center, 2300 Dayton Rd, Ames, IA, 50010, USA, Ph: 515-663-7455, Email: sbearson@nadc.ars.usda.gov; 1Iowa State University, Ames, IA, USA; 2USDA, ARS, Beltsville Agricultural Research Center, Beltsville, MD, USA

Abstract

The porcine response to infection with *Salmonella* is the result of differential expression of host-specific genes. To characterize these alterations in gene expression, functional genomic analyses were performed on swine tissues following experimental inoculation of the pigs with *Salmonella enterica* serovars Choleraesuis and Typhimurium. Suppression subtractive hybridization and quantitative real-time RT-PCR revealed that the transcriptional profiles of the porcine response to the swine-adapted strain (Choleraesuis) and the non-host-adapted strain (Typhimurium) exhibit unique differences. Our research demonstrates not only a difference in gene expression in animals infected with two different strains of *Salmonella* (host-specificity), but also alterations in the transcriptome over a time course of infection from the acute state (48 hr) to the chronic or carrier stage (21 days).

Introduction

The ability of higher animals to quickly and specifically alter their gene expression in response to pathogenic bacteria is an important mechanism to fight infection (Rosenberger et al., 2000; Strieter et al., 2002). Studies have documented differences in the immune responsiveness among animal breeds (Warner et al., 1987; Edfors-Lilja et al. 1998; Edfors-Lilja et al., 2000) and significant genetic control of disease resistance has been reported in pigs (Sellwood, 1979; Rothschild et al., 1984; Lunney and Butler, 1998; Meijerink et al., 2000; Lunney, 2003). Thus, understanding and improving the genetic control of disease resistance could benefit the swine industry by identifying useful tools for livestock breeding and production. Furthermore, since the transcriptional response mounted by the host’s immune system is highly pathogen-specific, profiling of the host immune gene expression is a recent revolutionary approach for diagnosing infectious diseases (Bodrossy and Sessitsch, 2004; Bryant et al., 2004; Campbell et al., 2004; Dawson et al., 2005).

As *Salmonella* is a foodborne pathogen that compromises animal health and food safety via its ability to cause intestinal and systemic disease, the objective of this research was to use a molecular approach to identify differentially expressed swine genes in response to *Salmonella* infection. The goals were to

1. investigate the host-specific response of *Salmonella*-infected pigs using two strains of *Salmonella*, a swine-adapted strain (Choleraesuis) and a generalist strain (Typhimurium) and
2. characterize the transcriptional profile of disease over a time course of infection from the acute to the chronic stage.

Understanding the immunological response of the pig and identifying the host signal(s) that confer resistance to *Salmonella* may provide targets for new drug discovery and diagnostic tests, improve intervention strategies, and/or pinpoint modifications in production practices to address pathogen load.

Materials and Methods

Conventionally raised piglets from *Salmonella* spp.-free sows were weaned at 10 days (d) of age, shipped to the NADC in Ames, IA and raised in isolation facilities. To confirm that all piglets were free of *Salmonella* prior to the experiment, bacteriological culturing was performed twice on rectal swabs. At 7 weeks of age, the pigs were divided into non-infected and infected groups with the infected groups receiving an intranasal challenge of 1 x 10⁹ CFU of either *S. Choleraesuis* χ3246 or *S. Typhimurium* χ4232. Rectal temperatures and clinical signs of infection (lethargy, loss of appetite and diarrhea) were recorded for each animal daily. At 8 hours (h), 24 h, 48 h, 7 d and 21 d post-infection (p.i.), three infected pigs were necropsied. Tissue samples from the mesenteric lymph nodes and lungs were aseptically collected and immediately frozen in liquid nitrogen for future mRNA isolation. Samples of the ileocecal lymph node were used for quantitative bacteriology.

Total RNA was prepared by homogenization of the frozen tissues in liquid nitrogen, extracted
using TRIzol reagent (Invitrogen), and purified using the RNeasy Midi kit and a RNase-free DNase set (Qiagen). Messenger RNA was isolated from total RNA using an Oligotex mRNA mini kit (Qiagen). The concentration and purity of the total RNA and mRNA was determined using the Agilent 2100 Bioanalyzer and the Genequant pro spectrophotometer (Amersham Biosciences).

Suppression subtractive hybridization was performed using the PCR Select cDNA Subtraction kit (Clontech). Forward and reverse subtractions were carried out using pooled mesenteric lymph node mRNA samples from three S. Choleraesuis and three S. Typhimurium infected pigs at 24 h post infection. Secondary (nested) PCR-amplified forward and reverse subtracted cDNA populations were cloned into the pBAD vector (pBAD TOPO TA Expression Kit, Invitrogen) and transformed into E. coli DH5α. Following overnight growth on selective media, random colonies were picked into 96-well plates for storage at -70°C.

Differential screening was performed on the subtracted clones to confirm their unique gene expression. The cDNA inserts were amplified by PCR using the following conditions: 95°C for 1 min followed by 30 cycles at 94°C for 30 sec, 68°C for 30 sec, 72°C for 1 min and a final cycle at 72°C for 5 min. PCR products were analyzed on 2% agarose gels to identify insert-containing clones. To prepare for Southern hybridization, 2 identical nylon membranes were made using the Bio-Dot apparatus (BioRad) by blotting 5 μl of denatured, PCR amplified cDNA inserts. For probe preparation, the forward and reverse subtracted secondary PCR products were digested with Rsa I to remove the adaptors then purified using QIAquick PCR purification kit (Qiagen). Probes (100 ng of denatured cDNA) were generated by alkaline phosphatase labeling with Gene Images AlkPhos Direct Labeling and Detection System (Amersham Biosciences). The two identical membranes were hybridized overnight at 55°C, one with the forward and the other with the reverse subtracted probes. The hybridization signals were visualized and quantified using a Multi-Imager Light Cabinet (Alpha Inotech Corporation). cDNA clones were considered differentially expressed when blots probed with the subtracted tester repeatedly demonstrated a signal intensity >1.5-fold different than blots probed with the subtracted driver. Plasmids containing differentially expressed cDNA were extracted and sequenced by dideoxy chain termination using an ABI 3700 DNA Analyzer (Applied Biosystems Inc.) at the Iowa State University DNA sequencing and synthesis facility. DNA homology searches were conducted using the Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) (Altschul et al., 1997). All selected plasmids were re-blotted in triplicate on nylon membranes and re-hybridized to forward and reverse subtracted secondary PCR products using the above described technique. Southern hybridization data was analyzed using the unpaired t test from GraphPad InStat software (GraphPad Software Inc).

Quantitative RT-PCR was performed to verify the differential expression of the clones of interest as well as measure specific immune marker gene expression (Dawson et al., 2005; Royaee et al., 2004). Using ABI PRISM 7700 and 7900 Sequence Detection Systems (Applied Biosystems), transcripts were amplified in triplicate and detected using either the QuantiTect SYBR Green RT-PCR kit (Qiagen) with amplification conditions of 50°C for 30 min, 95°C for 15 min, 35 cycles at 94°C for 15 sec, 55°C to 60°C for 30 sec (the annealing temperature varied depending on the gene specific primers), and 72°C for 5 sec or published procedures using the Stratagene Brilliant kit and 100 ng cDNA at 50°C for two min; 95°C for 10 min; 40 cycles of 95°C for 15 sec and 60°C for 1 min; then 4°C (Royaee et al., 2004; Dawson et al., 2005). Relative quantification of target gene expression was evaluated using cycle threshold (Ct) values. Gene expression data was normalized to the amount of RNA/cDNA amplified (Bustin, 2002). Fisher’s LSD post-hoc test was applied to assess differences between groups of pigs at different time points post infection. P <= 0.05 was considered statistically significant for all analyses.

Results As Salmonella spp have a large number of specific hosts, including birds, reptiles and mammals, Salmonella enterica serovars Choleraesuis (swine-adapted) and Typhimurium (generalist) were chosen to investigate Salmonella-host specificity in swine. Suppression subtractive hybridization was performed using pooled mRNA samples from the mesenteric lymph nodes of three S. Choleraesuis-infected pigs and three S. Typhimurium-infected pigs at 24 h p.i. Following differential screening by DNA blot hybridization, the DNA sequence was determined for 39 cDNA clones identified as induced in the S. Choleraesuis-infected pigs (compared to the S. Typhimurium-infected pigs) and 44 cDNA clones induced in the S. Typhimurium-infected pigs. Real-time RT-PCR
on select immune response genes (CXCL10, HSP105, SDCBP) validated the SSH results. Several of the genes identified in the SSH study were mapped to specific chromosomes and physical linkage positions using Radiation Hybrid mapping. Two of the genes, SDCBP and CXCL10, mapped within known quantitative trait loci (QTL) peaks for mitogen-induced proliferation and lymphocyte number, respectively (Edfors-Lilja et al. 1998; Edfors-Lilja et al., 2000).

The expression of a panel of immune genes was examined by real-time RT-PCR to further define the porcine response to Salmonella infection. S. Choleraesuis induced a more intense and extended up-regulation of porcine immune gene expression (IFNG, IL1B, IL6, TLR4) whereas S. Typhimurium triggered both a transient up-regulation (IFNG, SOCS1, STAT1) as well as a significant down-regulation in gene expression of many host immune factors (IL1B, IL4, IL6, TLR4, CSF2).

Discussion The clinical response elicited in swine infected with S. Choleraesuis is more severe than the clinical response to S. Typhimurium (Reed et al., 1986); furthermore, S. Choleraesuis usually does not progress into a carrier state in the host as observed with S. Typhimurium (Gray et al., 1996; Watson et al., 2000). The aim of our research was to investigate the molecular dynamics responsible for this difference in Salmonella-host specificity and characterize the porcine response during the various stages of Salmonella infection using functional genomic analyses. Suppression Subtractive Hybridization identified and real-time RT-PCR confirmed the differential expression of genes with a role in a variety of host cellular functions in response to Salmonella infection, including genes involved in innate immunity. Further genetic investigation revealed that two of the differentially expressed genes mapped to QTL sites for controlling mitogen-induced proliferation of whole blood cells (SDCBP) (Edfors-Lilja et al. 1998) and the number of circulating lymphocytes following mixing and transport of pigs (CXCL10) (Edfors-Lilja et al., 2000). CXCL10 expression has been shown to increase lymphocyte proliferation in the spleen in vitro (Whiting et al., 2004).

Therefore, this expression information will be useful in developing markers and screening for DNA polymorphisms associated disease response, potentially improving pig genetics.

As several immune-related genes were identified in the SSH analysis, specific immune genes were selected for transcriptional profiling using real-time RT-PCR. Unique differences were observed between the porcine gene expression responses to the two Salmonella strains: in general, a strong Th1 response (IFNG, INDO, STAT1) and expression of genes involved in inflammation and innate immunity (IL1B, IL6, TLR4) were observed in pigs inoculated with S. Choleraesuis. The pigs inoculated with S. Typhimurium exhibited a repression in Th2, innate and inflammatory response gene expression (IL1B, IL4, IL6, TLR4, CSF2) and only a transitory induction in the Th1 response (IFNG, SOCS1, STAT1).

Conclusions The porcine transcriptional responses during infection with S. Choleraesuis (swine-adapted) and S. Typhimurium (non-host-adapted) revealed unique host-specific differences. Although a response by the pigs to infection with both Salmonella strains was initiated by 24 hours p.i., the nature and persistence of that response was different between the two pathogens: S. Choleraesuis infection induced classic inflammatory gene expression, while S. Typhimurium induced a mild and transient transcriptional response of immune-related genes. These investigations suggest that S. Typhimurium may down-regulate the porcine immune response, thereby potentially evading aspects of the host’s immune system and progressing into a carrier state in the animal.

References


Dawson H.D., Beshah E., Nishii S., Solano-Aguilar G., Morimoto M., Zhao A., Madden K.B., Ledbetter T.K., Dubey J.P., Shea-


Lunney, J.K. 2003. Are there immune gene alleles that determine whether a pig will be healthy? Genetics of Pig Health Symposium, M. Boggess, Ed.; National Pork Board Press, Des Moines, IA. p.63-72


Sellwood R. 1979. Escherichia coli diarrhoea in pigs with or without the K88 receptor. Vet Rec 105: 228-30


