Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Gene Expressions in Major Organs between Enshi Black and Landrace Pigs after *Streptococcus suis* Infection

Uma Gaur[1,2§], Hua-Yu Wu[1,2§], Zheng Feng[1,2], Xue-Hai Yang[1,2], Rui Guo[1,2], Jun-Jing Wu[1,2], Kui Li[3], Mei-Lin Jin[4], Shu-Qi Mei [1,2] and Gui-Sheng Liu[1,2,*]

[1]Institute of Animal Science and Veterinary Medicine, Hubei Academy of Agricultural Sciences, Yaoyuan No. 1, Nanhu, Hongshan District, Wuhan 430064, China; [2]Hubei Key Laboratory of Animal Embryo Engineering and Molecular Breeding, Yaoyuan No. 1, Nanhu, Hongshan District, Wuhan 430064, China; [3]Beijing Institute of Animal Science and Veterinary Medicine, Chinese Academy of Agricultural Sciences, Malianwa, Haidian District, Beijing, 100193, China; [4]College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, Shizi Mountain No. 1, Nanhu, Hongshan District, Wuhan 430070, China
*Corresponding author: guisheng_liu1964@yahoo.com

ARTICLE HISTORY (14-046)

Received: January 26, 2014
Revised: March 14, 2014
Accepted: April 13, 2014

Key words:
Enshi black and Landrace pigs
Gene expression
Immune response
Streptococcus suis

ABSTRACT

Streptococcus suis 2 (*SS2*) is an important zoonotic agent, and its pathogenesis has been extensively studied recently. Our earlier study showed that there were different responses between pig breeds after *SS2* infection, which is similar to the previous observation in two mice lines. Bases on our previous transcriptome sequencing profiles, 15 target genes in spleen, lymph node, brain, lung, and liver were compared between Enshi black and Landrace breeds after *SS2* infection, by qPCR in this study. Gene *Resistin* was up-regulated in lymph and spleen of both breeds, whereas it was significantly down-regulated in lung and liver of Enshi black. *IL-1b* and *CD300c* was significantly up-regulated in the brain and lymph of Enshi black and the liver of Landrace, whereas *CXCL10* was significantly up-regulated in the brain and liver of Enshi black, and the lymph of Landrace pig. Gene *NPG3* was highly induced in the brain and lymph of Enshi black, but not so high in Landrace. *S100A8*, *S100A9* and *S100A12* were significantly increased in the brain of Enshi black but *S100A9* was significantly decreased in the lymph node of Enshi. *MMP9* in the brain was up-regulated in Enshi black whereas it was down-regulated in Landrace. We observed differential performances from 5 immunoglobulin genes, especially *IGLV5* and *IGKV6*. Our results suggested that Enshi black and Landrace have significant differences of innate and adaptive immune responses after *SS2* infection. The results could lay foundation for further deep study of resistance/ susceptibility mechanism in pigs.

To Cite This Article: Gaur U, HY Wu, Z Feng, XH Yang, R Guo, JJ Wu, K Li, ML Jin, SQ Mei and GS Liu, 2014. Gene Expressions in major organs between Enshi black and Landrace pigs after *Streptococcus suis* infection. Pak Vet J, 34(4): 432-437.

INTRODUCTION

Streptococcus suis (*SS*) is a Gram- positive facultative anaerobe coccus. Out of a total of 35 serotypes, *SS2* is the most frequent type recovered from diseased pigs. This serotype causes serious infections in people working in swine industries. The clinical features associated with S*S2* are meningitis, arthritis, endocarditis, pneumonia and septicemia with sudden death. To date, the studies of *SS2* have mainly focused on pathological and vaccine development aspects of the pathogen (Fittipaldi *et al*., 2012; Qi *et al*., 2013). As the different mice lines showed significantly differential susceptibility to *SS2* infection (Dominguez *et al*., 2008), some candidate genes were identified (Rong *et al*., 2012). Breed-specific differential immune response has been observed in two phenotypically different pig breeds infected with *SS2* by our previous work: the Enshi black pigs were more susceptible than Landrace pigs. It is very well evident that various tissues are involved in immunity against invading bacteria. The expression analysis of candidate genes involved in susceptibility or resistance in different tissues could give an

[§]These authors contributed equally to this work.

important insight of the differential mechanisms of immunity. This study was designed with an aim to further characterize differential expression of 15 candidate genes (Table 1) in 5 tissues, which are spleen, lung, lymph, brain and liver from both breed pigs.

CD163 expression is strongly coordinated in both tissue specific and differentiation specific manner. It is a surface molecule expressed mostly on cells possessing substantial anti-inflammatory potential (Kowal *et al*., 2011), and is found to be one of the important genes in immune network with response to *Haemophilus parasuis* (*H. parasuis*) infection (Zhao *et al*., 2013). Similarly CD300 antigen-like family member C (*CD300c*) which is also a receptor molecule and is present on monocytes, neutrophils and some T and B lymphocytes, is shown to have inflammatory actions. Chemokine CXC ligand 10 (*CXCL10*) is a member of CXC family, also known as interferon-inducible protein 10 (*IP-10*). It is a 10 kDa protein which could selectively attract and activate lymphocytes and governs immune responses (Hu *et al*., 2013). Cathelicidins form a family of small host defence peptides (Ramanathan *et al*., 2002). *Protegrin-3* (*NPG3*) which belongs to a family of cathelicidins (anti-microbial peptides) shows significant activity against a variety of pathogens. *Resistin* (*RETN*), an adipocytokine, is an important inter-mediator of the immune response and inflammation (Lago *et al*., 2007). Interleukin 1-beta (*IL-1b*) is one pro-inflammatory cytokine which has been an important contributor in the inflammation and pathogenesis (Christiansen *et al*., 2013; Hulst *et al*., 2013). Three genes from S100A gene family (*S100A8*, *S100A9* and *S100A12*) are also involved in immune response to the bacterial infection (Chen *et al*., 2009). Matrix metalloproteinase 9 (*MMP9*) supervises the activity of many cytokines and chemokines by cleavage and is related with tissue remodeling and regeneration as an after-effect of its ability to cleave structural extra-cellular matrix molecules (Hong *et al*., 2010; Vandooren *et al*., 2013). *S100A8* and *S100A9* have been proved to be the substrates for *MMP9* (Greenlee *et al*., 2006). The key role of *MMP9* in both initiation and completion of the inflammatory response is well contemplated. The population diversity of porcine antibody repository is greatly expanded because of the immense allelic variations found within the antibody light chain loci (Dawson *et al*., 2013). Immunoglobulins (Ig) are glycoprotein molecules which are produced by plasma cells in response to an immunogen and function as antibodies; bind particularly to one or few closely related antigens containing a specific antigenic determinant. They are composed of two identical light (23kD), and heavy chains (50-70kD). Ig can be classified into kappa and lambda on the basis of light chains that they have. The locus coding the porcine lambda light chain (IGL) contains 22 *IGLV* gene segments, out of which, 9 are functional. The IGL locus is organized into two distinct constant clusters on SSC14: a proximal cluster which contains *IGLV3* family members and a distal cluster containing *IGLV5* and *IGLV8* members (Schwartz *et al*., 2012a). The kappa light chain (IGK) locus is located on SSC3, comprised of at least 14 *IGKV* genes, with 9 functional genes and belong to either the *IGKV1* or *IGKV2* gene families, 5 *IGKJ* genes and a single *IGKC* gene (Schwartz *et al*., 2012b).

MATERIALS AND METHODS

Animal infection experiment, sample collection, RNA isolation and quality control: 12 pigs at age 7-8 weeks, of each Enshi black and Landrace pig breed were assigned randomly into two groups as the infected and the control group, with 6 in each. Pigs in the infected group were injected intravenously with *SS*2 strain SC19 with 1 ml of 94×10^5 colony-forming units (CFU). Each pig in the control group was treated with an identical volume of 0.9% NaCl. The temperature and the behavior changes of the pigs were noted at every 2 hours from the challenge to the sample collection. At 56h after challenge, necropsy was done and their spleen, brains, lungs, liver, and mandibular lymph nodes were collected and frozen immediately in liquid nitrogen with RNase-free equipment for further RNA analysis. Total RNA was extracted from approximately 200 mg of each sample using the TRIzol (Invitrogen) and purified by RNeasy Midi kit (QIAGEN) based on the manufacturer's protocols and the integrity, quality and quantity of the RNA samples were measured using the Agilent Bioanalyser 2100 (Agilent Technologies, Massy, France) and agarose gel electrophoresis.

Quantitative RT-PCR (qPCR): To compare the gene expression changes in 5 different tissues of both pig breeds, we performed qPCR on 15 candidate genes (Table 1) selected from spleen RNA-seq results. The first-strand cDNA synthesis was performed with superscript II reverse transcriptase (Invitrogen). Gene-specific primers were designed using primer premier 5.0, see Table 1. The *GAPDH* gene was used as an internal control. qPCR was carried out in triplicates containing SYBR Green (Bio-Rad) on Bio-Rad system using the following program: 95°C for 5 min; 35 cycles of 95°C for 30 sec, 60°C for 45 sec, and 72°C for 45 sec; 72°C for 7 min. All the tested genes are shown in Table 1. Gene expression level was calculated using the comparative Ct method (Pfaffl, 2001). The triplicates for each reaction were averaged. Fold changes (FC) value were calculated $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = (Ct_{test\ gene}-Ct_{test\ GAPDH}) - (Ct_{control\ gene}-Ct_{control\ GAPDH})$. *t* test was performed to calculate statistical significance of gene expression between the test group and control group. The FC≥2 and *p* value ≤0.05 were considered to indicate up-regulation; FC≤0.5 and P≤0.05 were considered to represent down-regulation. Genes, with FC between 0.5 and 2, were considered to show no significant change.

RESULTS

Clinical signs: The temperature and other clinical signs were observed carefully in all the experimental piglets at various time points. We observed different clinical symptoms between two pig breeds, and between the control and infected animals in each breed. Six SS2 infected Landrace piglets showed slight symptoms of anorexia, whereas the control Landrace piglets displayed a normal clinical behavior throughout all time points. Enshi black piglets started displaying differential clinical signs within 4 hours of inoculation and got differential degree of diarrhea, dyspnea, cramps, vomiting, ataxia, and lassitude and limb paralysis at many time points. One of the infected Enshi black piglets died at 52 hours of SS2 inoculation. A normal

clinical behaviour was observed in the control Enshi black piglets at all the time points. The bacteria were found in all the tissues in the infected animals from both breeds. (Gaur *et al*. 2014, unpublished data).

Resistin: Significant up-regulation of *RETN* gene is observed in lymph and spleen of both breed pigs in our experiment (Table 2), whereas significant down-regulation is seen in the lung (FC: 0.01, P=4.0E-04) and liver (FC: 0.05, P=9.0E-04) of Enshi black as well as in the lung tissue of Landrace pig (FC: 0.41, P=4.0E-04).

Cytokines and chemokines in inflammation: Cytokine *IL-1b* showed the maximum expression in Enshi-black brain (Table 2), whereas it is significantly down regulated in Landrace brain. Enshi black exhibited the continuous up-regulation of *IL-1b* in spleen, liver, lymph, and brain, except lung (Table 2). On the other hand, pro-inflammatory cytokine *IL-1b* is up-regulated only in spleen tissue in Landrace, down regulated in lymph and brain tissues and non-significant in liver and lung. The higher expression of the *CXCL-10* is reported in brain and liver of susceptible Enshi black pig, along with up-regulation in lymph of Landrace and non-significant in other tissues (Table 2).

Expression analyses of receptor molecules: CD163: *CD163* gene expression is dramatically consistently high in all tissues of Enshi black susceptible pig (Table 2), and down regulated in lung and brain of Landrace pig. CD300c: A down-regulated response is seen in all the tissue samples except liver of *SS2*-resistant Landrace pigs (Table 2), whereas a considerable up-regulation is observed in brain and lymph of Enshi black pigs, with a fold change of 31.77 (P=1.0E-03) and 5.31 (P=1.0E-04), respectively.

Cathelicidins: Protegrin *NPG3* expression was found to be highly induced in the brain and lymph of susceptible Enshi black pig (Table 2) with a FC of 152 (*p*-value: 8.0E-04) and 243 (P=5.0E-04) fold, whereas non-significant in Landrace pig, with up-regulation in spleen and lymph (FC: 5 fold each with P=3.97E-07 and 1.0E-04, respectively).

Calcium binding proteins and Matrix metallo-proteinase 9 expression: All three calcium binding protein genes were up-regulated in spleen of both pig breeds, with an exception of Enshi black in which *S100A9* was within the normal limits of expression i.e., FC: 1.77 (non-significant). Interestingly these three genes are dramatically induced in the brain of Enshi black, whereas normal expression in Landrace. *S100A9* and *S100A12* are up-regulated in lymph tissues of both breeds (Table 2).

MMP9 is found to be remarkably up-regulated in the spleen of resistant pig, and moderately up-regulated in the brain of Enshi black, whereas non-significant and moderately down-regulated in other tissues, respectively. In the spleen of Enshi black pig the expression of *MMP9* gene is within the normal limits (FC: 0.53, P=3.0E-03), which provided an interesting insight of porcine immune response (Table 2).

Immunoglobulins: *IGLV5* is up-regulated in the spleen and lymph of both breed pigs, whereas up-regulated in both liver and lung tissues in Landrace and non-significant expression of Enshi black (Table 2). The brain tissue is showing interesting expression profile of *IGLV5* with down-regulation in Landrace (FC: 0.0024, P=5.0E-04) and up-regulation in Enshi black (FC: 10.78, P=2.0E-03). *IGLV7* exhibited up-regulation in all the tissues except brain in the Landrace; whereas found up-regulated only in the spleen of Enshi black. For *IGLV8*, up-regulation is seen in spleen, lymph and liver of Landrace, and in spleen and brain of Enshi black pig, respectively; down-regulation is observed in the brain of Landrace and the lung of Enshi black. The *IGKV3* is dramatically induced during the *SS2*

Table 1: Information of 15 candidate genes chosen in the study

Gene name	Function	Primer sequences	Amplicon length
CXCL10	Chemokine	F- GGCCCTGCTACTGACACTTT R- GCAGTAGAAGCCCACGGAGT	171bp
NPG3	Microbicidal activity	F- TAGGTTCTGCGTCTGTGTCG R- AAATCCTTCACCGTCTACCAG	164bp
CD163	Cellular receptor	F- AAATGTGGAGTTGCCCTTTCTA R- ATGTGCTTCTCAGTCCCAGTG	104bp
CD300c	CMRF 35 antigen (CLN-6)	F- AGCCCCAGAAAAGCAGAAGT R- ATCTCTCCAGCACGGATGTC	110bp
IL-1b	Cytokine	F- CCTCTCCAGCCAGTCTTCAT R- TGTCACCGTAGTTAGCCATCA	176bp
RETN	Inflammation	F- TCCCTCCTCTTCCTCCCAAC R- CCAGTGACAGCAAAGCCTGA	200bp
IGLV8	Immunoglobulin	F- CGGCGATGTCAGTGTCTCCT R- GGCGGTTGTTTGTGCTGTAGAT	138bp
IGLV7	Immunoglobulin	F- CGGCGATGTCAGTGTCTCCT R- GGGCGGTTGTTTGTTCTGTAG	148bp
IGKV6	Immunoglobulin	F- CCAGATGACCCAGTCTCCAG R- TCCCTGCTTGTTGTTGATACC	119bp
IGKV3	Immunoglobulin	F- ACAGCTCCTGATCTATGGTGGT R- AGTAGTAAACGCCTGCATCCTC	131bp
IGLV5	Immunoglobulin	F- GCCTCGTAGACAGTGATGGAGA R- AGAATAAGAGCCTTGGCGACTG	78bp
MMP9	Remodelling of extracellular matrix	F- AACACACACGACATCTTCCAGTA R- AAGGTCACGTAGCCCACATAGT	119bp
S100A9	S100 calcium binding protein	F- CCAGGATGTGGTTTATGGCTTTC R- CGGACCAAATGTCGCAGA	186bp
S100A8	S100 calcium binding protein	F- GCGTAGATGGCGTGGTAA R- GCCCTGCATGTGCTTTGT	155bp
S100A12	S100 calcium binding protein	F- GGCATTATGACACCCTTATC R- GTCACCAGGACCACGAAT	168bp

Table 2: Fifteen candidate genes expression pigs in 5 tissues of Landrace (LD) and Enshi black (EB)

Gene	Spleen		Liver		Lymph		Lung		Brain	
	FC_ LD	FC _EB	FC_ LD	FC _EB	FC_ LD	FC _EB	FC_ LD	FC _EB	FC_ LD	FC _EB
CXCL10	0.18 ↓ (1.0E-02)	0.91 (8.0E-02)	0.17 ↓ (7.0E-04)	9.44 ↑ (2.6E-02)	2.37 ↑ (5.2E-05)	1.37 (4.0E-03)	0.07 ↓ (2.72E-05)	0.66 (1.2E-01)	0.08 ↓ (2.0E-04)	26.35 ↑ (6.0E-04)
NPG3	5.42 ↑ (4.0E-07)	0.32 ↓ (2.0E-03)	0.84 (3.0E-04)	1.98 (7.0E-04)	5.42 ↑ (1.0E-04)	243.87 ↑ (5.0E-04)	0.08 ↓ (6.4E-05)	2.58 ↑ (1.0E-03)	0.55 (4.0E-03)	152.21 ↑ (8.0E-04)
CD163	0.75 (1.0E-03)	6653.97 ↑ (1.0E-03)	0.73 (2.0E-04)	5712.87 ↑ (1.2E-05)	0.56 (1.2E-01)	9140.13 ↑ (2.7E-05)	0.31 ↓ (1.77E-05)	3420.52 ↑ (1.0E-04)	0.22 ↓ (3.0E-04)	48.5 ↑ (2.4E-05)
CD300c	0.19 ↓ (2.4E-05)	0.73 (5.0E-02)	0.51 (1.3E-01)	1.07 (3.0E-04)	0.29 ↓ (2.0E-03)	5.31 ↑ (1.0E-04)	0.08 ↓ (6.1E-05)	1.47 (1.4E-02)	0.36 ↓ (8.6E-05)	31.77 ↑ (1.0E-03)
IL-1b	5.9793 ↑ (4.0E-03)	2.69 ↑ (1.0E-03)	0.93 (5.0E-02)	2.88 ↑ (4.0E-02)	0.08 ↓ (2.2E-03)	5.89 ↑ (8.0E-04)	1.31 (1.0E-03)	1.76 (9.0E-03)	0.02 ↓ (5.0E-02)	83.86 ↑ (6.6E-05)
RETN	3.65 ↑ (3.0E-03)	2.62 ↑ (2.0E-04)	1.31 (1.0E-03)	0.05 ↓ (9.0E-04)	4.5 ↑ (1.0E-03)	17.87 ↑ (3.0E-04)	0.41 ↓ (4.0E-04)	0.01 ↓ (4.0E-04)	1.2 (5.0E-02)	0.82 (2.0E-04)
IGLV5	2.53 ↑ (1.4E-02)	4.82 ↑ (2.0E-04)	2.2 ↑ (2.0E-03)	1.05 (1.0E-04)	12.64 ↑ (7.0E-04)	2.54 ↑ (2.0E-04)	5.88 ↑ (6.2E-05)	1.4 (1.0E-03)	0.0024 ↓ (5.0E-04)	10.78 ↑ (2.6E-03)
IGLV7	8.87 ↑ (4.0E-04)	4.37 ↑ (2.0E-04)	15.24 ↑ (4.0E-04)	0.92 (5.0E-04)	72.5 ↑ (3.1E-05)	0.8 (7.0E-04)	4.56 ↑ (2.4E-05)	0.18 ↓ (1.0E-04)	0.17 ↓ (4.0E-04)	1.5 (1.9E-02)
IGLV8	4.02 ↑ (1.1E-02)	4.16 ↑ (1.4E-02)	3.75 ↑ (3.0E-03)	0.95 (1.0E-4)	12.64 ↑ (7.0E-04)	0.8 (1.7E-02)	1.03 (3.0E-04)	0.24 ↓ (1.6E-03)	0.03 ↓ (5.0E-04)	2.57 ↑ (8.0E-04)
IGKV3	205.07 ↑ (1.0E-04)	3.38 ↑ (1.3E-02)	76.63 ↑ (3.0E-03)	0.75 (2.0E-04)	13587.57 ↑ (1.0E-03)	1.56 (5.0E-04)	685.01 ↑ (2.0E-03)	0.29 ↓ (3.0E-04)	0.000031 ↓ (9.0E-04)	0.5 (2.0E-03)
IGKV6	0.07 ↓ (7.9E-05)	2.96 ↑ (4.0E-04)	0.07 ↓ (2.0E-03)	0.63 (5.0E-03)	0.26 ↓ (1.0E-03)	1.62 (4.0E-03)	0.03 ↓ (2.22E-05)	0.42 ↓ (4.6E-01)	0.002 ↓ (6.0E-04)	14.22 ↑ (5.0E-04)
MMP9	83.29 ↑ (4.0E-03)	0.53 (3.0E-03)	2.39 ↑ (4.0E-02)	0.73 (6.0E-04)	1.4 (1.0E-04)	0.67 (4.5E-02)	0.69 (1.2E-02)	0.45 ↓ (3.6E-01)	0.29 ↓ (1.0E-03)	4.56 ↑ (2.0E-03)
S100A9	27.47 ↑ (9.0E-03)	1.7654 (1.1E-01)	0.62 (5.3E-01)	0.06 ↓ (2.8E-02)	6.77 ↑ (5.0E-04)	477.71 ↑ (1.0E-04)	0.27 ↓ (5.0E-04)	0.29 ↓ (5.0E-02)	1.19 (1.1E-02)	3666.02 ↑ (2.5E-05)
S100A8	24.93 ↑ (4.0E-03)	2.17 ↑ (7.0E-03)	0.23 ↓ (6.0E-03)	1.48 (7.0E-03)	0.92 (3.0E-04)	0.07 ↓ (3.7E-06)	0.99 (3.0E-04)	0.63 (5.0E-03)	1.4 (3.0E-03)	989.11 ↑ (3.3E-05)
S100A12	35.27 ↑ (1.0E-03)	3.36 ↑ (2.9E-05)	0.41 ↓ (4.1E-02)	15.56 ↑ (4.0E-03)	5.89 ↑ (2.6E-05)	288.01 ↑ (2.0E-04)	0.61 (1.0E-04)	0.63 (4.0E-02)	0.86 (8.0E-02)	5873.48 ↑ (3.0E-05)

Values in bracket represent P-values. Fold change (FC) ≥2 and P≤0.05 were considered to indicate significant up-regulation; FC ≤0.5 and P≤0.05 were considered to represent significant down-regulation. FC between 0.5 and 2 were considered non-significant. ↑- indicated up-regulation; ↓- indicated down-regulation.

infection in all the tissues of Landrace except the brain tissue, where it is expressed at extremely low level. Up-regulation for *IGKV3* is detected only in spleen tissue of Enshi black. Significant down-regulation is noticed in all the tissues of Landrace for *IGKV6* (Table 2); whereas up-regulation is witnessed for the same gene in spleen and brain of Enshi black.

DISCUSSION

The significant differences in clinical signs were observed between the infected and control animals as well as between the 2 breeds. These observations lead us to the conclusion that there exists a differential immune response between these two pig breeds.

RETN has been recognized to act as a pro-inflammatory cytokine in humans. Johansson *et al*. (2009) demonstrated that the systemic *RETN* levels in septic shock differ depending on the causative micro-organisms. The *RETN* expression levels in lymph and spleen of both breed pigs indicates towards its important role in the immune response.

In mice, *S. suis* has induced high levels of pro-inflammatory cytokines and chemokines including *IL-1b* and *CXCL10* (Dominguez *et al*., 2008; 2010). In our study too we found interestingly similar results, this could possibly explain the serious meningitis and death caused to the Enshi-black pigs in our experiments. *IL-1b*-mediated leukocyte recruitment and other inflammatory events lead to neuronal cell death in some animal models. The relatively controlled expression of *IL-1b* in Landrace pigs indicated towards the role of anti-inflammatory cytokines in the control of inflammation.

Endogenous pro-inflammatory cytokines i.e. tumor necrosis factor-alpha (*TNF-a*), *IL-1a*, *IL-1b* or chemokines such as *CXCL-8* (also *IL-8*) decrease the expression of *CD163* (Sulahian *et al*., 2000). This inverse pattern of *IL-1b* and *CD163* expression is quite evident in our results.

As we can see that in the brain tissue of Enshi black pig, *IL-1b* expression is the highest and at the same time the *CD163* expression in brain is the lowest in comparison to other tissues analyzed. This directly validates the findings by (Sulahian *et al*., 2000), and gives a strong basis to the hypothesis, that controlled expression of pro-inflammatory cytokines can actually reduce the severity of pathogenesis, by controlling the inflammation.

The immune system is tightly regulated by a balance between activating and inhibitory signals in order to provide an adequate response to eliminate the pathogens, while preserving the host self. The *CD300* molecule expression is altered by bacterial products and pro-inflammatory cytokines and in return *CD300* molecules alters the uptake of molecules, secretion of pro-inflammatory cytokines and migration of cells, which is an indispensable part of immunity and regulation of inflammatory responses (Borrego, 2013). The expression pattern of *CD300c* in Landrace and Enshi black pig points towards its important role in inflammation. The high expression of protegrin *NPG3* in brain and lymph of Enshi black pig is consistent with clinical signs in *SS2* infected pigs.

The calcium binding protein genes belong to the S100 family, which are also known as myeloid-related proteins (MRPs), play a pivotal role in the innate immune system and have been considered as efficient clinical markers of inflammation (Foell *et al*., 2007). At the time of infection,

cell damage or inflammation, these intracellular molecules become pro-inflammatory danger signals after release into the extracellular compartment (Chen *et al*., 2009; Foell *et al*., 2007). Chen *et al*. (2009) reported the following fold changes in *H. parasuis* infected porcine spleen: *S100A8*: 8.4 fold, *S100A9*: 21.8 fold and *S100A12*: 16.6 fold, whereas in our qPCR analysis the same genes are remarkably up-regulated in spleen, lymph and brain of Enshi black. In concordance with the previous reports the expression of these three calcium binding proteins partly explained the severe inflammation observed in Enshi black pigs in the present study.

The interesting observation is the normal expression of *MMP9* gene in the spleen of susceptible Enshi black pig which is contrasting to the results by Rong *et al*. (2012), where the *MMP9* gene expression in *SS2*-infected A/J mice was increased by 7.04 fold, while *SS2*-infected B6 mice displayed a fold change of only 1.99. This is to be noted that A/J mice are significantly more susceptible than C57BL/6 (B6) mice to *SS2* infection. This makes *MMP9* an ideal target for further deep gene analysis, to find out the role of *MMP9* in *SS2* pathogenesis in swine.

Apart from the genes responsible for inflammatory response, we have profiled the expression of 5 antibody producing immunoglobulin genes. Two genes of Ig Kappa variable *IGKV3* and *IGKV6*, and 3 genes of Ig lambda variable *IGLV5*, *IGLV7* and *IGLV8* are compared for differential gene expression in different tissues from two breed pigs. Previous report by Schwartz *et al*., (2012a) suggested that *IGLV* expression is restricted to *IGLV3* and *IGLV8*, but in our results we found that *IGLV5* is also used in pre-immune repertoire. The significantly higher expression of *IGKV3* in resistant Enshi black pig makes this gene an important candidate for further deep study.

We can clearly see a difference in expression of antibody specific genes in two differentially susceptible pig breeds, which indicate towards the underlying differences in genetic make-up of resistant and susceptible animals.

Conclusion and perspective: We reported differential performances of 15 genes in 5 tissues; further molecular level exploration is required to open up the complex mechanism of resistance or susceptibility to a pathogen. Continuous exposure to the harsh environment and disease stimulus also makes an organism more resistant towards some pathogens, which is most likely due to the epigenetic control of immune response **(**Bierne *et al*., 2012). The deep study of different genetic controls operating behind the immune responses of two phenotypically differentially susceptible pig breeds could delineate the differential mechanisms of immunity to *SS2*, i.e. putative promoter regions, 3'UTR's and epigenetic controls. This could give an overall picture of the molecular resistance or susceptibility shown by the organism and help to design the better breeding strategies and more effective controls for *SS2* infection.

Acknowledgement: This work was supported by national key project for breeding of new transgenic varieties (2009ZX08009-141B) and national post-doctor foundation (2012M511596) and innovation system of agricultural science and technology in Hubei Province (2011-620-001-003) and open project from Hubei Key Laboratory for Animal Embryo Engineering and Molecular Breeding (2011ZD102).

REFERENCES

Bierne H, M Hamon and P Cossart, 2012. Epigenetics and Bacterial Infections. Cold Spring Harb Perspect Med, 2: a010272.

Borrego F, 2013. The CD300 molecules: an emerging family of regulators of the immune system. Blood, 121: 1951-1960.

Chen H, C Li, M Fang, M Zhu, X Li, R Zhou, K Li and S Zhao, 2009. Understanding *Haemophilus parasuis* infection in porcine spleen through a transcriptomics approach. BMC Genomics, 10: 64.

Christiansen IG, HE Jensen, LK Jensen, J Koch, B Aalbaek, OL Nielsen and PS Leifsson, 2013. Systemic inflammatory response and local cytokine expression in porcine models of endocarditis. APMIS, 122: 292-300.

Dawson HD, JE Loveland, G Pascal, JGR Gilbert, H Uenishi, KM Mann, Y Sang, J Zhang, DC Silva, T Hunt, M Hardy, Z Hu, S Zhao, A Anselmo, H Shinkai, C Chen, B Badaoui, D Berman, C Amid, M Kay, D Lloyd, C Snow, T Morozumi, RP Cheng, M Bystrom, R Kapetanovic, JC Schwartz, R Kataria, M Astley, E Fritz, C Steward, M Thomas, L Wilming, D Toki, AL Archibald, B Bed'Hom, D Beraldi, T Huang, T Ait-Ali, F Blecha, S Botti, TC Freeman, E Giuffra, DA Hume, JK Lunney, MP Murtaugh, JM Reecy, JL Harrow, CR Gaillard and CK Tuggle, 2013. Structural and functional annotation of the porcine immunome. BMC Genomics, 14: 332.

Dominguez-Punaro MC, MM Segura, D Radzioch, S Rivest and M Gottschalk, 2008. Comparison of the susceptibilities of C57BL/6 and A/J mouse strains to *Streptococcus suis* serotype 2 infection. Infect Immunity, 76: 3901-3910.

Dominguez-Punaro MC, MM Segura and I Contreras, 2010. *In Vitro* Characterization of the Microglial Inflammatory Response to *Streptococcus suis*, an Important Emerging Zoonotic Agent of Meningitis. Infect Immunity, 78: 5074-5085.

Fittipaldi N, M Segura, D Grenier and M Gottschalk, 2012. Virulence factors involved in the pathogenesis of the infection caused by the swine pathogen and zoonotic agent *Streptococcus suis*. Future Microbiol, 7: 259-279.

Foell D, H Wittkowski, T Vogl and J Roth, 2007. S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. J Leukoc Biol, 81: 28-37.

Greenlee KJ, DB Corry, DA Engler, RK Matsunami, P Tessier, RG Cook, Z Werb and F Kheradmand, 2006. Proteomic identification of *in vivo* substrates for matrix metalloproteinases 2 and 9 reveals a mechanism for resolution of inflammation. J Immunol, 177: 7312-7321.

Hong JS, K Greenlee, SH Lee, LZ Song, M Shan, R Pitchumani, SH Chang, PW Park, A Bidani, C Dong, Z Werb, D Corry and F Kheradmand, 2010. Requirement of MMP2 and MMP9 for lung innate immune defense against *Streptococcus pneumoniae* infection. J Immunol, 184: 37.34.

Hu PF, XC Li, N Lei, XY Lan, QJ Zhao, WJ Guan and YH Ma, 2013. Study on characteristics of chemokine CXCL10 gene cloned from cDNA expression library of Ujumqin sheep. BioMed Res Int, doi: 10.1155/2013/217942.

Hulst M, M Smits, S Vastenhouw, AD Wit, T Niewold and JVD Meulen, 2013. Transcription networks responsible for early regulation of Salmonella-induced inflammation in the jejunum of pigs. J Inflamm, 10: 18.

Johansson L, A Linner, J Sunden-Cullberg, A Haggar, H Herwald, K Loré, CJ Treutiger and A Norrby-Teglund, 2009. Neutrophil-Derived Hyper-resistinemia in Severe Acute Streptococcal Infections. J Immunol, 183: 40.

Kowal K, R Silver, E Sławinska, M Bielecki, L Chyczewski and O Kowal-Bielecka, 2011. CD163 and its role in inflammation. FHC, 49: 365-374.

Lago F, C Dieguez, J Gómez-Reino and O Gualillo, 2007. Adipokines as emerging mediators of immune response and inflammation. Nat Clin Pract Rheumatol, 3: 716-24.

Pfaffl MW, 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res, 29: e45.

Qi ZT, JY Tian, QH Zhang, R Shao, M Qiu, ZS Wang, QJ Wei and JT Huang, 2013. Susceptibility of Soiny Mullet (*Liza haematocheila*) to *Streptococcus dysgalactiae* and physiological response to formalin inactivated *S. dysgalactiae*. Pak Vet J, 33: 234-237.

Ramanathan B, EG Davis, CR Ross and F Blecha, 2002. Cathelicidins: microbicidal activity, mechanisms of action, and roles in innate immunity. Microbes Infect, 4: 361-372.

Rong J, W Zhang, X Wang, H Fan, C Lu and H Yao, 2012. Identification of Candidate Susceptibility and Resistance Genes of Mice Infected with *Streptococcus suis* Type 2. PLoS One, 7: e32150.

Schwartz JC, MP Lefranc and MP Murtaugh, 2012a. Organization, complexity and allelic diversity of the porcine (*Sus scrofa domestica*) immunoglobulin lambda locus. Immunogenetics, 64: 399-407.

Schwartz JC, MP Lefranc and MP Murtaugh, 2012b. Evolution of the porcine (*Sus scrofa domestica*) immunoglobulin kappa locus through germline gene conversion. Immunogenetics, 64: 303-311.

Sulahian TH, P Hogge, AE Wahner, K Wardwell, NJ Goulding, C Sorg, A Droste, M Stehling, PK Wallace, PM Morganelli and PM Guyre, 2000. Human monocytes express CD163, which is upregulated by IL-10 and identical to p155. Cytokine, 12: 1312-1321.

Vandooren J, VD Steen and G Opdenakker, 2013. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): The next decade. Crit Rev Biochem Mol Biol, 48: 222-272.

Zhao M, X Liu, X Li, H Chen, H Jin, R Zhou, M Zhu and S Zhao, 2013. Systems infection biology: a compartmentalized immune network of pig spleen challenged with *Haemophilus parasuis*. BMC Genomics, 14: 46.