GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

TLR4 gene expression in pig populations and its association with resistance to *Escherichia coli* F18

J. Wang[1], Z.Y. Pan[1], X.R. Zheng[1], Z.C. Wu[1], X.M. Su[1], G.Q. Zhu[2], X.G. Huang[3], S.L. Wu[1] and W.B. Bao[1]

[1]College of Animal Science and Technology,
Key Laboratory for Animal Genetics, Breeding,
Reproduction and Molecular Design of Jiangsu Province,
Yangzhou University, Yangzhou, China
[2]College of Veterinary Medicine, Yangzhou University, Yangzhou, China
[3]Suzhou Taihu Pig Breeding Center, Jiangsu Province, Suzhou, China

Corresponding authors: W.B. Bao / S.L. Wu
E-mail: wenbinbao74@yahoo.com / pigbreeding@163.com

Genet. Mol. Res. 12 (3): 2625-2632 (2013)
Received August 7, 2012
Accepted March 15, 2013
Published July 30, 2013
DOI http://dx.doi.org/10.4238/2013.July.30.1

ABSTRACT. *TLR4* is the main recognition receptor of bacterial lipopolysaccharides, which play an important role in innate and adaptive immunity. We used real-time PCR to analyze the tissue expression profile and differential expression of *TLR4* in 4 pig populations (*Escherichia coli* F18-resistant Sutai, *E. coli* F18-sensitive Sutai, Large White, Meishan), in order to determine the role that the *TLR4* gene plays in resistance to *E. coli* F18. We found that *TLR4* expressed consistently in the 4 populations, with relatively high levels in immune tissues and the highest level in the lung. Generally, the expression of *TLR4* in *E. coli* F18-sensitive individuals was the highest, followed by that in *E. coli* F18-resistant, Large White and Meishan. In the spleen, lung, kidney, lymph nodes, and thymus gland, *TLR4* expression is significantly higher in the *E. coli* F18-sensitive than in the other 3 populations; there were no significant differences among *E. coli* F18-resistant Sutai, Large White, and Meishan. In addition, Gene Ontology and pathway analysis showed that *TLR4* takes part in the inflammatory response. We found that porcine *TLR4* has consistent tissue specificity in each breed, and downregulation of expression of the *TLR4*

gene is related to resistance to *E. coli* F18 in weaning piglets.

Key words: *Escherichia coli* F18; Pig; Real-time PCR; *TLR4* gene

INTRODUCTION

To date, the post-weaning diarrhea or edema disease caused by *Escherichia coli* F18, a type of Gram-negative bacteria, has caused great economic losses in the pig industry. Lipopolysaccharides (LPS) from *E. coli* F18 can be recognized by Toll-like receptor 4 (*TLR4*). However, the relationship between *E. coli* F18 disease and porcine *TLR4* expression is still unclear.

In recent years, much research has focused on the genetic variations of *TLR4*, part of which has reported that the gene is involved in susceptibility and resistance to diseases to a certain extent (Leveque et al., 2003). However, studies on the expression of *TLR4* affected by LPS in various cells have emerged in multitude, showing that LPS can upregulate the expression of *TLR4* (Yang et al., 2010; Guzzo et al., 2012), as well as showing phyletic specificity and histiocyte-specificity. However, the impacts of *TLR4* are like a double-edged sword. On one hand, *TLR4* signaling in macrophages activates hundreds of genes that contribute to defense against infection by bacteria, such as *Mycobacterium tuberculosis* (Abel et al., 2002), *Pneumococcal* (Klein et al., 2008), and *Salmonella* (Roy et al., 2006). On the other hand, by initiating the secondary inflammatory signaling pathways, *TLR4* can amplify acute inflammatory responses and cause severe disease, such as atopic dermatitis (Penders et al., 2010) and acute lung injury (ALI) (Wang et al., 2011). Therefore, the role of *TLR4* is so important in disease. Although the mechanism is still unknown, the levels of *TLR4* expression can play a crucial role in it. Therefore, detecting *TLR4* expression can be important in further research on the mechanism of *TLR4*.

In general, *TLR4* is widespread in T lymphocytes, B lymphocytes, leukocytes, macrophages, intestinal epithelial cells, and respiratory epithelial cells (Zhang et al., 2005) and expressed at a low level in the intestinal mucosa (Abreu et al., 2001; Otte et al., 2004). However, research on the expression of *TLR4* in swine is scarce, with only some reports showing a high level in lung (Qiu et al., 2007). Considering that the resistance to pathogens varies between foreign and Chinese domestic breeds, the examination of their *TLR4* expression is of great significance.

As a key linkage, *TLR4* plays a very important role between natural and specific immunity. The level of *TLR4* expression is closely connected with the resistance to disease. Considering the initial studies on the *TLR4* gene, the detection of *TLR4* gene expression in foreign, hybrid, and Chinese domestic pig breeds is an attractive route for the analysis of differences in genetic resistance and mechanisms with regard to *E. coli* F18 infection in 3 pig breeds (Large White, Sutai, and Meishan).

The pathogenesis of F18 antigen is that after *E. coli* strains enter the pig's intestinal tract, they strongly adhere to intestinal epithelial cells via their pili and bind to the F18 receptors in the brush border membrane of intestinal epithelial cells. They then settle, propagate, produce enterotoxins, and cause diseases in piglets (Bertin and Ducher-Suchaux, 1991; Nagy and Fekete, 1999). Using candidate gene approach and linkage analysis, Vogeli et al. (1997) demonstrated that alpha (1,2)-fucosyltransferase gene (*FUT1*) on chromosome 6q11 is a candidate gene controlling the adhesion to F18 receptor. The research of Meijerink et al. (1997) showed that there is a G/A mutation at position M307 of the *FUT1* gene, with G being predominant over A. In other words, pigs with genotype AA are resistant to ETEC F18 and pigs with genotype GG or the heterozygote AG are sensitive to ETEC F18. Breeding for disease resistance could be implemented by marker-assisted selection using the *FUT1* gene as the ETEC F18R candidate gene. Using a small number of

FUT1 AG type (9.2%) individuals detected from Sutai pigs (Bao et al., 2008), our group conducted proper selection and assortative mating. Small intestine epithelial cells of resistant (AA genotype) and sensitive (AG and GG genotypes) pigs were selected to test the adhesion capability of the wild-type *E. coli* expressing F18ab fimbriae, the recombinant *E. coli* expressing F18ac fimbriae, or the recombinant *E. coli* secreting and surface-displaying the FedF subunit of F18ab fimbriae, respectively (Wu et al., 2007). After 5 years of breeding, we now established resistant (AA genotype) and sensitive (AG and GG genotypes) resource populations to ETEC F18 in our Sutai pig population. Thereby, Sutai pig can be considered as the linkage between foreign and domestic breeds in the fields of investigating *TLR4* gene expression and *E. coli* F18 infection in pigs. The expression of the *TLR4* gene in different pig breeds can be also helpful in the development of research on the functions of the *TLR4* gene and its potential relationship with resistance to *E. coli* F18.

MATERIAL AND METHODS

Experimental materials and sample collection

Large White, Sutai, and Meishan pigs (35 days old) were obtained from the Engineering Research Centre for Molecular Breeding of Pig in Changzhou City of Jiangsu Province, *E. coli* F18-resistant/sensitive population in Suzhou Taihu Pig Breeding Center, and Meishan Pig Conservation Breeding Company, respectively. Each population included 8 piglets. After sacrifice, heart, liver, spleen, lung, kidney, stomach, thymus, lymph node, jejunum, duodenum, and muscle were collected in 1.5-mL Eppendorf nuclease-free tubes, stored immediately in liquid nitrogen, and then placed in a low-temperature freezer (-80°C) until further study.

Real-time PCR primer design

Using the Primer Express 2.0 software, *TLR4* primers were designed on the basis of the sequence of AB232527 (http://www.ncbi.nlm.nih.gov/) in GenBank and synthesized by Shanghai Invitrogen Biotechnology Co., Ltd. *GAPDH* was used as an internal control to normalize all of the threshold cycle (Ct) values of other tissue products. Primer sequences for amplification of *TLR4* and *GAPDH* are listed in Table 1.

Table 1. Primers used for real-time PCR.

Gene	Sequence	Expected length (bp)
TLR4	Forward primer: 5'-CAGATAAGCGAGGCCGTCATT-3' Reverse primer: 5'-TTGCAGCCCACAAAAAGCA-3'	113
GAPDH	Forward primer: 5'-ACATCATCCCTGCTTCTACTGG-3' Reverse primer: 5'-CTCGGACGCCTGCTTCAC-3'	187

RNA extraction and reaction system and conditions for fluorescence quantitative PCR

Total RNA was extracted from various swine tissues (50-100 mg) using Trizol reagent (TaKaRa Biotechnology Dalian Co., Ltd.). Precipitated RNA was resuspended in 20 μL RNase-free H_2O and then stored at -80°C. RNA quality and quantity were assessed by agarose gel electrophoresis and UV spectrophotometer, respectively.

The 10-µL reaction mixture for cDNA synthesis contained the following: 2 µL 5X PrimerScript Buffer, 0.5 µL PrimerScript RT Enzyme Mix I, 0.5 µL Oligo dT, 0.5 µL random 6-mers, 500 ng total RNA, and RNase-free H_2O to make up the final volume of 10 µL. The reaction was carried out at 37°C for 15 min and then at 85°C for 5 s.

Real-time PCR amplification was performed in a 20-µL reaction mixture containing 1 µL cDNA (100-500 ng), 0.4 µL 10 µM of each forward and reverse primer, 0.4 µL 50X ROX Reference Dye II, 10 µL 2X SYBR Green Real-Time PCR Master Mix, and 7.8 µL ddH_2O. PCR conditions were 95ºC for 15 s, followed by 40 cycles of 95ºC for 5 s and 62ºC for 34 s. The dissociation curve was analyzed after amplification. A peak of Tm at 85 ± 0.8°C on the dissociation curve was used to determine the specificity of PCR amplification. The Tm value for each sample was the average of the real-time PCR data for triplicate samples.

Data processing and analysis

The $2^{-\Delta\Delta Ct}$ method was used to process the real-time PCR results (Livak and Schmittgen, 2001). Statistical analyses were carried out using the SPSS 15.0 software (SPSS Inc, Chicago, IL, USA), and a *t*-test was carried out to determine the significance of differences in mRNA expression between different breeds.

RESULTS

The purity and integrity of total RNA

Total RNA samples were assayed using 1% agarose gel electrophoresis. Three bands, representing 28S, 18S, and 5S, were observed with no bands from DNA contamination or significant degradation. RNA purity was also examined on a UV spectrophotometer. The A_{260}/A_{280} ratios of the samples were 1.8-1.9.

Fluorescence quantitative PCR amplification curve and melting curve

The PCR amplification curve and the dissociation curve for the *TLR4* gene showed good repetition, and a single specific peak was observed with the real-time PCR products for the *TLR4* gene with no primer dimers or nonspecific reaction products. The standard curves for the *TLR4* and *GAPDH* genes indicated that the amplification efficiencies of the target gene and the reference gene were almost the same, so that the $2^{-\Delta\Delta Ct}$ method could be applied for quantitative calculation (Figure S1). Data were analyzed by SPSS 11.5 and shown as means ± SD. The *t*-test was used to inspect the significance of expression in different populations.

Results of *TLR4* gene expression in different tissues and populations

Using the established SYBR green real-time quantitative PCR method described above, the expression levels of *TLR4* were examined in various tissues in this study. The expression level of *TLR4* in the heart of the resistance group was defined as 1.0. As the results shown in Table 2 and Figure S2, the *TLR4* gene was expressed in all tissues tested with the highest level in lung. In general, there was high expression in immune tissues, including thy-

Table 2. Differentiation of *TLR4* mRNA expression between different pig populations.

Tissue (number)	Heart	Liver	Spleen	Lung	Kidney	Stomach	Muscle	Lymph nodes	Thymus gland	Duodenum	Jejunum
Resistant Sutai pig (8)	1.000 ± 0.201	17.350 ± 3.290	119.512 ± 29.640^{b}	207.270 ± 46.562^{b}	26.844 ± 9.364^{b}	28.051 ± 9.722	0.252 ± 0.189	19.581 ± 3.180^{b}	28.278 ± 7.188^{b}	15.683 ± 6.155^{ab}	8.440 ± 1.310
Sensitive Sutai pig (8)	1.741 ± 0.217	18.792 ± 3.984	161.210 ± 32.891^{a}	275.369 ± 52.650^{a}	42.765 ± 9.426^{a}	23.356 ± 9.297	0.662 ± 0.347	42.248 ± 5.101^{a}	84.280 ± 8.660^{a}	31.432 ± 10.203^{a}	10.011 ± 1.513
Large White (8)	0.936 ± 0.279	10.149 ± 3.124	114.212 ± 20.925^{b}	170.811 ± 20.894^{b}	25.347 ± 11.608^{b}	23.072 ± 14.098	0.738 ± 0.299	17.972 ± 8.820^{b}	27.481 ± 5.813^{b}	13.529 ± 3.426^{b}	4.951 ± 2.283
Meishan (8)	1.261 ± 1.061	6.825 ± 4.567	95.106 ± 17.255^{b}	145.636 ± 20.143^{b}	24.201 ± 12.187^{b}	32.814 ± 17.945	1.731 ± 0.872	14.855 ± 4.899^{b}	22.292 ± 3.671^{b}	14.434 ± 4.048^{b}	13.541 ± 9.196

Means with the different superscript letters within the same column differ significantly ($P < 0.05$).

mus gland, lymph node, and spleen. In addition, the expression of the *TLR4* gene was the highest in sensitive Sutai, followed by resistant Sutai, Large White, and Meishan pigs. There was a consistent expression profile in the tissues of spleen, lung, kidney, lymph nodes, and thymus gland, in which *TLR4* expression in *E. coli* F18-sensitive individuals was significantly higher than that in other breeds ($P < 0.05$). In duodenum, the expression of the *TLR4* gene in *E. coli* F18-sensitive ones was significantly higher than that in Large White and Meishan ($P < 0.05$). There was no significant relationship between other tissues in the 4 populations.

TLR4 Gene Ontology (GO) and pathway analysis

According to GO database classifications generated by the US National Center for Biotechnology Information (NCBI) and the KEGG database (http://www.genome.ad.jp/kegg/), the main function of the *TLR4* gene has been determined. As shown in Table S1, *TLR4* takes part in 97 biological function processes, mainly integral to membranes, and is involved in the immune response as well as response to LPS. In addition, *TLR4* participates in 15 pathways, such as Toll-like receptor signaling pathway, pertussis, and tuberculosis (Table S2). The results showed that it had the potential to participate in 3 pathways related to recognition of Gram-negative bacteria, where this important information could be used as a basis for further study.

DISCUSSION

Medzhitov et al. (1997) first identified and cloned the human *TLR4* gene, which has a higher degree of expression in spleen, endothelial cells, macrophages, neutrophils, and dendritic cells. In swine, Bao et al. (2011) reported that *TLR4* expressed in all tissues tested, with the highest expression in lung and a relatively high expression level in immune tissue. In this study, *TLR4* expressed in all tissues among 3 breeds with the highest expression level in the lungs and a relatively high level in spleen, kidney, thymus, lymph tissue, and the results were in agreement with Bao et al. (2011). The results showed that there was a consistent tissue expression pattern in Sutai, Meishan, and Large White, indicating that porcine *TLR4* has consistent tissue specificity in each breed.

This study showed that in high *TLR4* expressing tissues of Sutai such as lung, spleen, kidney, lymph node, and thymus, the expression of the *TLR4* gene in *E. coli* F18-sensitive individuals was significantly higher than that in resistant individuals ($P < 0.05$), indicating that *TLR4* has a certain impact on porcine Gram-negative bacteria such as *E. coli* F18. Thus, we can draw the conclusion that upregulation of *TLR4* expression can increase the risk of infection by *E. coli* F18. On the other hand, the results indicated that downregulation of *TLR4* expression may be related to resistance to *E. coli* F18. On the basis of the GO and KEGG databases, the results of functional analysis of the *TLR4* gene implied more detailed relationships between *TLR4* and *E. coli* F18, such as response to bacteria, inflammatory response, and LPS receptor activity.

Current reports show that individuals with *TLR4* gene deficiency or lower expression have a lower rate of infection caused by pathogens (Oyama et al., 2004; Van Linthout et al., 2011). However, some reports show that antigen peptide from pathogens such as LPS can stimulate dendritic cells to overexpress *TLR4* (An et al., 2002), which will cause continued expansion of inflammation by activating NF-κB (Bauer et al., 2007). It is worth discussing what is the kind of causal relationship between the upregulation of *TLR4* gene expression and

E. coli infection. It is still unknown whether it is overexpression of *TLR4* that decreases the body's resistance to *E. coli*, or the *E. coli* infection that induces *TLR4* gene expression, making the body more sensitive to *E. coli*. The next step is to analyze the relationship between *TLR4* gene and *E. coli* systematically.

Some previous studies showed that the downregulation of *TLR4* expression was closely associated with human disease resistance, including cancer, pulmonary disease, and dermatitis diseases (Wieland et al., 2005; Sun et al., 2008; Penders et al., 2010), which implied that the downregulation of *TLR4* was related to general resistance. In addition, some research has also shown that Chinese indigenous pig breeds such as Meishan, one of the materials of this study, have a strong general resistance (He et al., 2001; Clapperton et al., 2005). In this study, the lower expression of *TLR4* in the vast majority of Meishan pigs further implied that the downregulation of *TLR4* expression had a correlation with general resistance.

Compared to *E. coli* F18-sensitive Sutai groups, *TLR4* mRNA was downregulated in *E. coli* F18-resistant ones in the lung, spleen, kidney, lymph nodes, and thymus. In addition, it is worthwhile noting that there were no differences between *E. coli* F18-resistance and the other 2 breeds Large White and Meishan, which showed that when screening the disease-resistance groups, it is impossible to improve the body's inherent general resistance just by focusing on a special pathogen such as *E. coli* F18. *TLR4*, as a recognition of antigen, not only can trigger innate immunity but can also activate specific immunity, acting as a bridge in the key link connecting innate immunity with specific immunity (Beutler, 2005). These test results suggested that the *TLR4* gene may be closely associated with the genetic basis of species innate immune response. Aiming at a specific pathogen for breeding by molecular methods cannot improve the species' innate immune response. There is a need for further analysis of the innate immune pathways and immune regulatory mechanism to provide guidance and basis for improving the body's immune response.

ACKNOWLEDGMENTS

Research supported by the National Natural Science Funds (#31172183), the Prospective Joint Research Project of Jiangsu Province (#BY2012157), the Science and Technology Supporting Project of Jiangsu Province (#BE2012330), and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Supplementary material

REFERENCES

Abel B, Thieblemont N, Quesniaux VJ, Brown N, et al. (2002). Toll-like receptor 4 expression is required to control chronic *Mycobacterium tuberculosis* infection in mice. *J. Immunol.* 169: 3155-3162.

Abreu MT, Vora P, Faure E, Thomas LS, et al. (2001). Decreased expression of Toll-like receptor-4 and MD-2 correlates with intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide. *J. Immunol.* 167: 1609-1616.

An H, Yu Y, Zhang M, Xu H, et al. (2002). Involvement of ERK, p38 and NF-kappaB signal transduction in regulation of *TLR2*, *TLR4* and *TLR9* gene expression induced by lipopolysaccharide in mouse dendritic cells. *Immunology* 106: 38-45.

Bao WB, Wu SL, Musa HH, Zhu GQ, et al. (2008). Genetic variation at the alpha-1-fucosyltransferase (*FUT1*) gene in Asian wild boar and Chinese and Western commercial pig breeds. *J. Anim. Breed. Genet.* 125: 427-430.

Bao WB, Ye L, Pan ZY, Zhu J, et al. (2011). Analysis of polymorphism in the porcine *TLR4* gene and its expression related

to *Escherichia coli* F18 infection. *Czech J. Anim. Sci.* 56: 475-482.
Bauer S, Hangel D and Yu P (2007). Immunobiology of toll-like receptors in allergic disease. *Immunobiology* 212: 521-533.
Bertin AM and Duchet-Suchaux MF (1991). Relationship between virulence and adherence of various enterotoxigenic *Escherichia coli* strains to isolated intestinal epithelial cells from Chinese Meishan and European large white pigs. *Am. J. Vet. Res.* 52: 45-49.
Beutler B (2005). The Toll-like receptors: analysis by forward genetic methods. *Immunogenetics* 57: 385-392.
Clapperton M, Bishop SC and Glass EJ (2005). Innate immune traits differ between Meishan and Large White pigs. *Vet. Immunol. Immunopathol.* 104: 131-144.
Guzzo C, Ayer A, Basta S, Banfield BW, et al. (2012). IL-27 enhances LPS-induced proinflammatory cytokine production via upregulation of *TLR4* expression and signaling in human monocytes. *J. Immunol.* 188: 864-873.
He WM, Jiang YF, Luo WH, Huang JW, et al. (2001). Pathogenic diagnosis and epidemiological investigation on edema disease of pigs. *Acta Agric. Bor.-Occid. Sin.* 10: 1-5.
Klein M, Obermaier B, Angele B, Pfister HW, et al. (2008). Innate immunity to *Pneumococcal* infection of the central nervous system depends on toll-like receptor (TLR) 2 and *TLR4*. *J. Infect. Dis.* 198: 1028-1036.
Leveque G, Forgetta V, Morroll S, Smith AL, et al. (2003). Allelic variation in *TLR4* is linked to susceptibility to *Salmonella enterica* serovar Typhimurium infection in chickens. *Infect. Immun.* 71: 1116-1124.
Livak KJ and Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* 25: 402-408.
Medzhitov R, Preston-Hurburt P and Janeway CA Jr (1997). A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* 388: 394-397.
Meijerink E, Fries R, Vogeli P, Masabanda J, et al. (1997). Two α(1,2) fucosyltransferase genes on porcine chromosome 6q11 are closely linked to the blood group inhibitor (S) and *Escherichia coli* F18 receptor (ECF18R) loci. *Mamm. Genome* 8: 736-741.
Nagy B and Fekete PZ (1999). Enterotoxigenic *Escherichia coli* (ETEC) in farm animals. *Vet. Res.* 30: 259-284.
Otte JM, Cario E and Podolsky DK (2004). Mechanisms of cross hyporesponsiveness to Toll-like receptor bacterial ligands in intestinal epithelial cells. *Gastroenterology* 126: 1054-1070.
Oyama J, Blais C Jr, Liu X, Pu M, et al. (2004). Reduced myocardial ischemia-reperfusion injury in toll-like receptor 4-deficient mice. *Circulation* 109: 784-789.
Penders J, Thijs C, Mommers M, Stobberingh EE, et al. (2010). Host-microbial interactions in childhood atopy: toll-like receptor 4 (*TLR4*), *CD14*, and fecal *Escherichia coli*. *J. Allergy Clin. Immunol.* 125: 231-236.
Qiu XT, Li YH, Li HJ, Yu Y, et al. (2007). Localization and tissue expression of swine Toll-like receptor 4 (*TLR4*). *J. Agr. Biotechnol.* 15: 37-40.
Roy MF, Lariviere L, Wilkinson R, Tam M, et al. (2006). Incremental expression of *Tlr4* correlates with mouse resistance to *Salmonella* infection and fine regulation of relevant immune genes. *Genes Immun.* 7: 372-383.
Sun Q, Zheng Y, Liu Q and Cao X (2008). Rapamycin reverses *TLR4* signaling-triggered tumor apoptosis resistance by disrupting Akt-mediated Bcl-xL upregulation. *Int. Immunopharmacol.* 8: 1854-1858.
Van Linthout S, Spillmann F, Graiani G, Miteva K, et al. (2011). Down-regulation of endothelial *TLR4* signalling after apo A-I gene transfer contributes to improved survival in an experimental model of lipopolysaccharide-induced inflammation. *J. Mol. Med.* 89: 151-160.
Vogeli P, Meijerink E, Fries R, Neuenschwander S, et al. (1997). A molecular test for the detection of *E. coli* F18 receptors: a breakthrough in the struggle against edema disease and post-weaning diarrhea in swine. *Schweiz. Arch. Tierheilkd.* 139: 479-484.
Wang Q, Zhu H, Zhu J and Zhou LN (2011). Dynamic expression of Toll-like receptor 4 in septic acute lung injury in rats. *J. Chin. Prac. Diagand. Ther.* 25: 157-161.
Wieland CW, Florquin S, Maris NA, Hoebe K, et al. (2005). The MyD88-dependent, but not the MyD88-independent, pathway of *TLR4* signaling is important in clearing nontypeable *Haemophilus influenzae* from the mouse lung. *J. Immunol.* 175: 6042-6049.
Wu SL, Yuan ZW, Ju HP, Huang XG, et al. (2007). Polymorphisms of the *FUT1* gene M307 locus in post-weaning Sutai breed piglet and resistance to F18 fimbrial *Escherichia coli in vitro*. *Chinese J. Prev. Vet. Med.* 29: 783-787.
Yang H, Wei J, Zhang H, Song W, et al. (2010). Upregulation of Toll-like Receptor (TLR) expression and release of cytokines from mast cells by IL-12. *Cell Physiol. Biochem.* 26: 337-346.
Zhang X, Shan P, Qureshi S, Homer R, et al. (2005). Cutting edge: *TLR4* deficiency confers susceptibility to lethal oxidant lung injury. *J. Immunol.* 175: 4834-4838.