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Gene expression profiles in the intestine of lipopolysaccharide-challenged piglets

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TABLE OF CONTENTS

1. ABSTRACT

Bowel diseases are common in human and animals and are characterized by intestinal dysfunction and injury. A well-established porcine model of intestinal injury can be induced by lipopolysaccharide (LPS), an endotoxin released from the cell wall of pathogenic bacteria. LPS affects the expression of genes associated with intestinal immune response, mucosal growth, energy metabolism, absorption, mucosal barrier function, and antiviral function. Transcriptional analysis of intestinal genes reveals that the duodenum, jejunum, ileum and colon respond to LPS challenge in a similar pattern. Moreover, the jejunum and ileum exhibit greater responses to LPS challenge than the duodenum and colon with regard to gene expression. Additionally, over 85% of genes are co-expressed along the small and large intestines and there is a clear distinction in gene expression patterns amongst the different intestinal segments in pigs. These findings have important implications for underlying molecular mechanisms responsible for endotoxin-induced intestinal injury and dysfunction.

2. INTRODUCTION

Weanling piglets are beset with psychological, nutritional, and environmental stresses, and are readily attacked by pathogenic bacteria, resulting in intestinal mucosal barrier dysfunction and diarrhea. This not only causes economical losses in swine production, but also contributes to public health risk from pathogenic bacteria-infected pork products. Typically, enteropathogenic *Escherichia coli* (*E. coli*) is a diarrhea-causing bacterium that can disrupt the intestinal function directly by binding cell-surface molecules, inducing changes in expression of tight junction proteins (1) and activating mucosal and systemic inflammatory responses (2).

Lipopolysaccharide (LPS) is an endotoxin released from the cell wall of pathogenic bacteria and is well known as an immune stressor in pigs (3-5), broilers (6,7), rodents (8,9), and humans (2). LPS causes various morphologic changes in the intestine, such as villus atrophy, submucosal edema, epithelial vacuolation, and necrosis (10). Moreover, LPS results in intestinal dysfunction (11) and increase mucosal permeability (12). Our previous studies also showed that LPS administration affected intestinal energy metabolism and stimulated inflammatory responses (13,14). However, the molecular mechanisms responsible for LPS-mediated alterations of intestinal function are not fully understood. In the present article, we highlight recent studies regarding gene expression patterns in the small and large intestines of LPS-challenged piglets.

3. INTESTINAL RESPONSE TO LIPOPOLYSACCHARIDE CHALLENGE

3.1. General study protocols

A well-established porcine model of intestinal injury induced by a LPS challenge was employed to determine the intestinal transcriptional responses to LPS (3,5). Pigs were weaned at 28 days of age to a corn- and soybean meal-based diet (5). After a 7-day period of adaptation, sixteen piglets (35 days of age, average body weight of 11.6 ± 0.26 kg) were assigned randomly into one of the two treatment groups: control group (non-challenged control, piglets received intraperitoneal administration of sterile saline) and LPS group (LPS-challenged group, piglets received intraperitoneal administration of *Escherichia coli* LPS). Each group had eight piglets. Piglets had free access to food and drinking water. The corn- and soybean meal-based diet was formulated to meet National Research Council (NRC, 2012)-recommended requirements for all nutrients. On days 1, 4, and 11 of the trial, overnight fasted piglets of the LPS group received intraperitoneal administration of LPS (*Escherichia coli* serotype 055: B5) at the dose of 100 μg/kg BW, whereas piglets in the control group received intraperitoneal administration of the same volume of sterile saline (13,14). To exclude a possible effect of LPS-induced reduction in food intake on piglet intestinal function, piglets in the control group were pair-fed the same amount of feed per kg body weight as that consumed by LPS piglets during the post-challenge period (days 1 to 11 of the trial). At 6 h post-administration of LPS or saline (day 11 of the trial), all piglets were killed under anesthesia with an intravenous injection of pentobarbital sodium (50 mg/kg BW) to obtain intestinal mucosal samples (13).

All assays were performed using the published methods (14,15). To determine intestinal morphology, four paraformaldehyde-fixed intestinal samples (duodenum, jejunum, ileum, and colon) were dehydrated and embedded in paraffin. Five-μm sections were cut and then stained with hematoxylin and eosin stain. Intestinal morphology was determined using a light microscope (Leica, Germany) with Leica Application Suite image analysis software (Leica, Germany).

The transcriptional response of the mucosae of small and large intestines to LPS were quantified using real-time RT-PCR (16,17). Approximately 100 mg of each frozen mucosal sample was powdered and homogenized, and total RNA was isolated using the Trizol Reagent protocol (Invitrogen, Carlsbad, CA). Total RNA was quantified using the NanoDrop® ND-1000A UV-VIS spectrophotometer (Thermo Scientific, Wilmington, DE, USA) at an OD of 260 nm, and its purity was assessed by determining the OD260/OD280 ratio (1.8 to 2.1). Total RNA was reverse-transcribed using a PrimeScript® RT reagent kit with gDNA Eraser (Takara, Dalian, China) according to the manufacturer's instruction. cDNA was synthesized and stored at -20 °C until use.

To amplify cDNA fragments, the primers pairs (Table 1) were used for qPCR. To minimize amplification of potentially contaminating genomic DNA, the primers were designed to span introns and intron-exon boundaries. The qPCR was performed using the SYBR® Premix Ex Taq™ (Takara, Dalian, China) on an Applied Biosystems 7500 Fast Real-Time PCR System (Foster City, CA). The total volume of PCR reaction system was 50 μL. In brief, the reaction mixture contained 0.2. μM of each primer, 25 μL of SYBR® Premix Ex Taq™ (2 ×) and 4 μL of cDNA in a 50-μL reaction volume. All PCRs were performed in triplicate on a 96-well real-time PCR plate (Applied Biosystems) under the following conditions (two-step amplification): 95 °C for 30 sec, followed by 40 cycles of 95 °C for 5 sec and 60 °C for 31 sec. A subsequent melting curve (95 °C for 15 sec, 60 °C for 1 min and 95 °C for 15 sec) with continuous fluorescence measurement was constructed, followed by setting at 25°C). The specificity of the qPCR reactions was assessed by analyzing the melting curves of the products and size verification of the amplicons (23). To ensure the sensitivity and accuracy of the results obtained by qPCR, samples were normalized internally using simultaneously the average cycle threshold (Ct) of ribosomal protein L4 (RPL4) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference genes in each sample to avoid any artifact of variation in the target gene (29,25). Results were analyzed by the $2^{-\Delta Ct}$ method (16). Each biological sample was run in triplicate.

All the data are expressed as means ± SD. According to the $2^{-\Delta Ct}$ method, the mean value of gene expression in the control group was set to 1.00. Similarly, the mean value of duodenal gene expression was set to 1.00 in piglets without LPS challenge. A fold-change less than 1 ($P < 0.05$) means down-regulation. Conversely, a fold change higher than 1 ($P < 0.05$) means up-regulation (4). Additionally, the normality and constant variance for all data were tested by the Levene's test (30). Differences among treatment means were determined by the Student's unpaired t-test. All statistical analyses were performed by the SPSS 13.0 software (Chicago, IL, USA). Possibility values < 0.05 were taken to indicate statistical significance (31).

3.2. LPS induces alterations of intestinal morphology

The morphological structure of the intestine is shown in Figure 1. The duodenum, jejunum, ileum, and colon of piglets in the control group (Figure 1a, c, e, and g) were normal in structure. However, in the LPS group, histomorphological abnormal changes associated with intestinal mucosal injury were observed. Specifically, the epithelium at the tips of the small intestinal villus (Figure 1b, d, and f) was shorter, and the

Table 1. Sequences of the primers used for quantitative RT-PCR analysis

Genes	Forward	Reverse	References or Genebank no.
EGF	GCCATAAGGGTGTCAGGTATTT	TGCTTTGCTCTTGCCCTCTAC	NM_214020
EGFR	GGCCTCCATGCTTTTGAGAA	GACGCTATGTCCAGGCCA A	NM-2140075
AR	GAGTACGATAACGAACCGCACA	TTTCCACTTTTGCCTCCCTTT	NM_214376
TGF-α	TGGAAAGCGGCAACCAA	GCCCGAGAGAGCAATACAGG	NM_214015
IGF-1	GCCCAAGGCTCAGAAGG	TTTAACAGGTAACTCGTGC	Reference 18
IGF-1R	ATTCGCACCAATGCTTCA	AGGGCGGGTTCCACTTC	Reference 18
IGFBP-3	TGACTCCAAACTCCACTC	TTGGACTCAGAGGAGAAG	Reference 19
mTOR	TTGTTGCCCCCTATTGTGAAG	CCTTTCGAGATGGCAATGGA	Reference 20
Ki-67	CGCAACCAAGCAAC	ACAGTGCCAAACTGGGAGAAA	NM_001101827
Caspase-3	GAACTCTAACTGGCAAACCCAAA	GTCCCACTGTCCGTCTCAATC	NM_214131
Occludin	TATGAGACAGACTACACAACTGGCGGCGAGTCC	ATCATAGTCTCCAACCATCTTCTTGATGTG	Reference 21
Claudin-1	GGTGCCCTACTTTGCTGCTC	CCCACACGGTTTTGTCCTTT	NM_001244539
SGLT-1	CCCAAATCAGAGCATTCCATTCA	AAGTATGGTGTGGTGGCCGGTT	Reference 22
pBD-1	ACCGCCTCCTCCTTGTATTC	CACAGGTGCCGATCTGTTTC	Reference 23
pBD-2	TTGCTGCTGCTGACTGTCTG	CTTGGCCTTGCCACTGTAAC	Reference 23
AQP-8	TGTGTCTGGAGCCTGCATGAAT	AGCAGGAATCCCACCATCTCA	NM_001112683
ZO-1	AGGCGATGTTGTATTGAAGATAAATG	TTTTTGCATCCGTCAATGACA	Reference 22
CFTR	CCGGGCACCATTAAAGAAAAC	GCCATCAATTTACAGACACAGC	Reference 24
Na^+/K^+-ATPase	TCGATAATCTCTGCTTCGTTGG	ATGGCTTTGGCTGTGATGG	NM_214249
REG3A	GAAGATTCCCCAGCAGACAC	AGGACACGAAGGATGCCTC	Reference 25
ALP	CCACTCCCACGTCTTTACCTTT	CTCTCACCACCCACCACCTT	AY145131
HSP70	GACGGAAGCACAGGAAGGA	GAAGACAGGGTGCGTTTGG	NM_001123127
AMPK	CGACGTGGAGCTGTACTGCTT	CATAGGTCAGGCAGAACTTGC	Reference 26
PGC-1α	GATGTGTCGCCTTCTTGTTC	CATCCTTTGGGGTCTTTGAG	Reference 27
Sirt1	CTGGAACAGGTTGCAGGAAT	CCTAGGACATCGAGGAACCA	Reference 27
UCP2	AGGGTCCCCGAGCCTTCT	CAGCTGCTCATAGGTGACAAACA	Reference 28
COX7A1	AAGAGGAGGACGCAGGATGA	CAGCCACTCGGTTCTCCAA	NM_214411
COX7C	CCGTAGGAGCCACTATGAGGAG	GCAGCAAATCCAGATCCAAAG	NM_001097474
TLR4	GCCTTTCTCTCCTGCCTGAG	AGCTCCATGCATTGGTAACTAATG	AB188301
NF-κB	CTCGCACAAGGAGACATGAA	ACTCAGCCGGAAGGCATTAT	DQ834921
IFN-α	ACTCCATCCTGGCTGTGAGGAAAT	ATCTCATGACTTCTGCCCTGACGA	NM214393
IFN-β	CAACGTGCAGTCTATGGAGT	GAGGTGCTGATGTACCAGTTG	GQ415073.1
MX1	AGTGCGGCTGTTTACCAAG	TTCACAAACCCTGGCAACTC	NM_214061.1
MX2	CGCATTCTTTCACTCGCATC	CCTCAACCCACCAACTCACA	NM_001097416.1
IL-1β	ATGTCAGAAGCTCCTGGGACAGTT	AGGTCATCCATCTGCCCATCAAGT	M86725
CXCL9	CTTGCTTTTGGGTATCATCTTCCT	TCATCCTTTGGCTGGTGTTG	NM_001114289.2
CXCL10	GGCTTCTACTGCTTTCCTTCCA	ATTTTCAGGGATTTCTAGCTTCTCC	NM_001008691.1
CXCL11	GCAGTGAAAGTGGCAGATATTGAG	TTTGGGATTTAGGCATCTTCGT	NM_001128491.1
OASL	GGCACCCCTGTTTTCCTCT	AGCACCGCTTTTGGATGG	NM_001031790.1

(*Contd*...)

Table 1. (***Continued***)

Genes	Forward	Reverse	References or Genebank no.
OAS1	TGGTGGTGGAGACACACACA	CCAACCAGAGACCCATCCA	NM_214303.1
OAS2	GTTGGAGATTCATGGACTGATATTG	AGAGAAGCAAGGGAAGAAGGAGTAG	NM_001031796.1
IFIT1	GCTAAACCAAACACCGCAGAA	GGAACTCAATCTCCTCCAAGACC	NM_001244363.1
IFIT2	CAGAAGGCGGCAGAGAATG	ACACAGAGGCAGGCGAGATAG	JX070599.1
IFIT3	GCATTTTCCAGCCAGCATC	TCTGTTCCTTTCCTTTCCTTCCT	NM_001204395.1
IFIT5	CAGAAAATACAGCCATCCACCA	AGGGCACTTAAACTCTGCACATC	XM_001925952.3
PKR	GAATTTTGGCACCTACATTTACCTC	CTTTCACTTCACGCTCTACCTTCTC	NM_214319.1
$b^{0,+}AT$	CGAGTACCCGTACCTGATGGA	TGCGTAGAAGGGCGAAGAA	NM_001110171.1
PepT1	ATTCTCAGGCTCCTTCCAACA	GCAACCCCGCAAACAGA	NM_214347.1
ASCT2	AAGATTGTGGAGATGGAGGATGT	CAGGAAGCGGTAGGGGTTT	DQ231578.1
SNAT2	AGAAAACCGAAATGGGAAGGT	CTTGCTTGGTGGGGTAGGAG	KC769999.1
GAPDH	CGTCCCTGAGACACGATGGT	CCCGATGCGGCCAAAT	AF017079
RPL4	GAGAAACCGTCGCCGAAT	GCCCACCAGGAGCAAGTT	Reference 25

crypt architecture was distorted in the colon (Figure 1h). Previous studies have shown that LPS administration resulted in not only the decrease of villus height and villus surface area, but also an increase of crypt depth (5,32), supporting the notion that LPS can induce intestinal morphologic alterations and injury. LPS is known to induce the intestinal inflammation and increase the levels of pro-inflammatory cytokines, such as IL-1, IL-6 and TNF-α (14,15), which may contribute largely to the intestinal injury. Of particular note, TNF-α has been shown to play an essential role in regulating intestinal epithelial cell apoptosis and/or survival and in serving as a mediator in the injury of gastrointestinal tract (33,34). In addition to the induction of intestinal morphologic alterations and injury, LPS was also reported to impair intestinal mucosal barrier function, induce oxidative stress, and alter intestinal energy status (14,32).

3.3. Quantification of gene expression in the intestine of piglets without LPS challenge

Over 85% of genes were co-expressed along the small and large intestines (35,36). However, there was a clear distinction in gene expression patterns amongst the different intestinal segments (36). As shown in Table 2, the mRNA levels for regenerating islet-derived protein 3 alpha (REG3A), heat shock protein 70 (HSP70), and sirtuin 1 (Sirt1) were highest in the duodenum, and those for occludin, sodium/glucose co-transporter 1 (SGLT-1), Na^+/ K^+-ATPase, and peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) were highest in the jejunum. Similarly, mRNA levels for mTOR, Ki-67, claudin-1, zonula occluden 1 (ZO-1), porcine β-defensins 1 (pBD-1), alkaline phosphatase (ALP), AMP-activated protein kinase (AMPK), and uncoupling protein 2 (UCP2) were most abundant in the ileum, whereas those for EGF, epidermal growth factor receptor (EGFR), amphiregulin (AR), IGF-1, and pBD-2 were most abundant in the colon. Other genes, including TGF-α, IGFBP-3, caspase-3, the cystic fibrosis transmembrane conductance regulator (CFTR), aquaporins 8 (AQP8), cytochrome c oxidase subunit VIIa 1 (COX7A1), and cytochrome c oxidase subunit VIIa 1 (COX7A1), were also differently expressed in the intestine (Table 2). Therefore, these genes, as listed in Table 2, were expressed with regional differences in the intestine, suggesting that the regulation of gene expression in the intestinal mucosae varies with different intestinal segments. For instance, genes, including REG3A, mTOR, Ki-67, and caspase-3, participated in the maintenance of enterocyte structure and function, as well as constant replacement of enterocytes by regeneration of epithelial cells from crypts and the constant production of protective mucusins in young pigs (37). Higher expression levels of these four genes are associated with growth and apoptosis of the upper intestine. Moreover, the small intestine is the major absorption site and, therefore, SGLT-1 and Na^+/ K^+-ATPase are more abundant in the duodenum.

3.4. LPS regulates the expression of genes associated with intestinal mucosal physiology

In pigs, EGFR and mTOR signaling pathways are crucial for enterocyte growth, proliferation and regeneration (38,39), and also play a vital role in the recovery of the small intestinal mucosa after damage (40,41). As reported previously (14,32), mRNA levels for EGF in the duodenum, jejunum, and colon and for mTOR in the jejunum and ileum were decreased by LPS challenge (Table 3). These results indicate that LPS challenge suppresses expression of genes associated

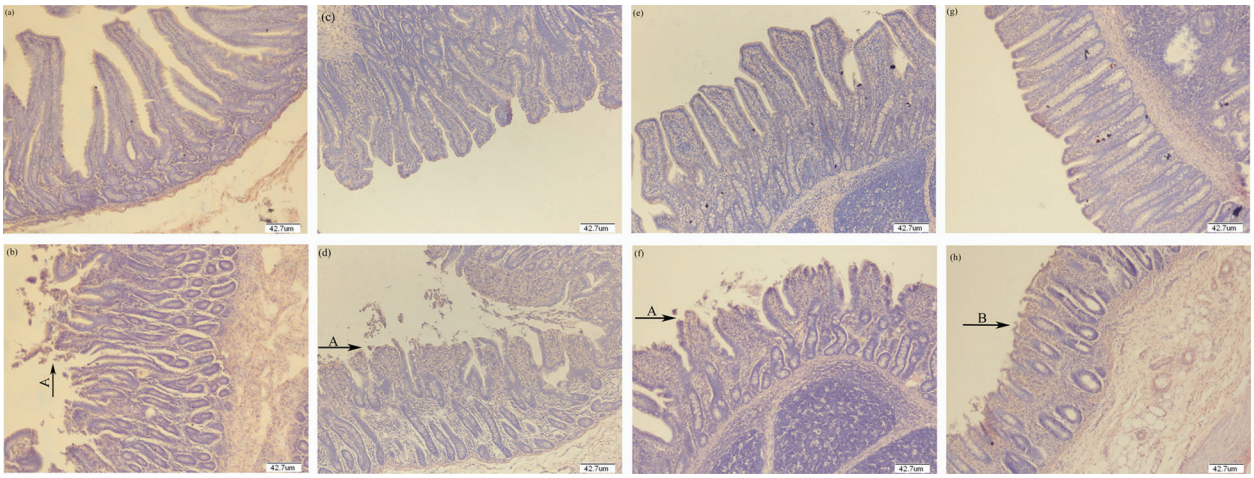

Figure 1. Morphological characterization of the small intestine and colon. Duodenum (a), jejunum (c), ileum (e), and colon (g) of piglets receiving intraperitoneal administration of sterile saline. No obvious changes in their morphology were observed. Duodenum (b), jejunum (d), ileum (f), and colon (h) of piglets challenged with E. coli LPS. Morphological changes associated with intestinal mucosal injury, such as lifting of the epithelium at the tip of the villus (A), and distorted colon crypt morphology (B). Sections were stained with hematoxylin and eosin. Original magnification 100 ×. Scale bar = 42.7 μm.

with protein synthesis and tissue repair. In addition, the LPS-induced intestinal injury may be associated with the IGF-1 signaling pathway. This view is substantiated by the finding that the IGF-1 mRNA level was decreased in the jejunum, ileum, and colon post administration of LPS. Interestingly, the mRNA level for Ki-67 was reduced only in the ileum but the mRNA level for caspase-3 was not altered in all intestinal segments of LPS-challenged piglets. Since the abundance of the caspase-3 protein is augmented in the small intestine of endotoxin-treated piglets (32), it is likely that LPS plays an important role in the regulation of intestinal caspase-3 expression primarily at the translational level.

Intestinal mucosal barrier function is closely related to the epithelial integrity which is maintained by cohesive interactions between cells via the formation of tight junctions (41). Many lines of evidence suggest that members of the claudin family of proteins play a vital role in tight junction formation and determination of permeability characteristics in the gut (42,43). LPS disrupts mucosal barrier function and increases paracellular permeability in a time- and dose-dependent manner through altering the abundance and function of tight junction proteins (occludin, claudin-1, and ZO-1) and reducing the expression of ZO-1 (44). In our study, we found that LPS challenge down-regulated expression of the genes for intestinal claudin-1 and occludin without affecting expression of the ZO-1 gene (Table 3). These results are in agreement with the previous study of Hou *et al*. (33), who reported that LPS challenge reduced the protein abundance of claudin-1 and occludin in the small intestinal mucosae of piglets. It is possible that gene transcription of intestinal claudin-1 and occludin is affected by LPS, which may result from the increases of TNF-α and IL-6 proteins in the jejunum of LPS-challenged piglets. TNF-α and IL-6, the central mediators of intestinal inflammatory diseases, decrease the amounts of tight junction proteins and increase intestinal permeability through dysregulation of claudin and occludin expression (45). AQP8, the major water transporter in the gastrointestinal tract (46), was significantly down-regulated in the intestinal mucosae of LPS-challenged piglets (Table 3). This result was in line with studies of Lehmann *et al*. (47) and Hou *et al*. (14). Impaired absorption of water by the intestine is expected to cause diarrhea and dehydration in animals.

Beta-defensins (BDs) are important antimicrobial peptides of the innate immune system and provide protection against bacterial infection in the intestine (48). In pigs, there were contradictory results of BD expression in response to infection. Veldhuizen *et al*. (48) observed an increase in expression of the pBD-1 and pBD-2 genes after *in vitro* infection of intestinal porcine epithelial cell lines with *S. typhimurium*. However, the same research group did not observe up-regulation of pBD-1 and pBD-2 *in vivo* in the small intestine upon infection with *S. typhimurium* (49). Collado-Romerro reported that pBD-2 was up-regulated in the ileum but down-regulated in the jejunum, whereas no changes in pBD-1 mRNA levels were observed after *in vivo* infection with *S. typhimurium* (4). In contrast, we observed that intestinal expression of the pBD-1 gene was up-regulated, but that of the pBD-2 gene was not affected, by LPS administration into piglets (Table 3). It can be surmised that pBD-1 plays a crucial role in protection against the LPS-induced intestinal stress. In addition, REG3A was down-regulated in the small intestine (particularly in the jejunum) of LPS-challenged piglets (50,51). The magnitude of the decrease in REG3gene expression suggests its important role in the innate defense against bacteria.

Table 2. Patterns of gene expression in the intestine of piglets without LPS treatment

Genes	Fold-change	SD	Fold-change	SD	Fold-change	SD	Fold-change	SD
EGF	1^{b}	0.13	0.27^{c}	0.06	0.72^{b}	0.14	6.93^{a}	1.31
EGFR	1^{b}	0.13	0.76^{c}	0.12	1.08^{b}	0.13	1.68^{a}	0.23
AR	1^{c}	0.26	2.80^{b}	0.33	2.99^{b}	0.20	6.52^{a}	1.37
TGF-α	1^{a}	0.02	0.91^{ab}	0.04	1.19^{a}	0.35	0.74^{b}	0.14
IGF-1	1^{d}	0.07	1.55^{c}	0.33	2.98^{b}	0.39	6.35^{a}	1.02
IGF-1R	1^{c}	0.04	1.63^{b}	0.19	2.24^{a}	0.37	0.75^{d}	0.14
IGFBP-3	1^{b}	0.26	1.88^{a}	0.35	1.11^{b}	0.16	1.67^{a}	0.19
mTOR	1^{b}	0.22	0.94^{b}	0.18	1.39^{a}	0.31	0.82^{b}	0.05
Ki-67	1^{c}	0.09	1.38^{b}	0.19	1.75^{a}	0.39	1.16^{bc}	0.13
Caspase-3	1^{b}	0.15	1.34^{a}	0.03	1.48^{a}	0.38	0.27^{c}	0.02
Occludin	1^{b}	0.15	3.15^{a}	0.51	0.43^{c}	0.06	0.68^{c}	0.15
Claudin-1	1^{b}	0.02	0.98^{b}	0.09	1.39^{a}	0.21	0.93^{b}	0.22
ZO-1	1^{b}	0.02	1.11^{ab}	0.19	1.33^{a}	0.12	0.82^{c}	0.09
AQP-8	1^{ab}	0.24	0.84^{b}	0.08	0.91^{b}	0.26	1.20^{a}	0.18
SGLT-1	1^{c}	0.10	4.07^{a}	0.93	2.32^{b}	0.49	0.07^{d}	0.02
CFTR	1^{a}	0.13	0.93^{a}	0.05	0.93^{a}	0.29	0.63^{b}	0.15
Na^{+}/K^{+}-ATPase	1^{b}	0.12	1.80^{a}	0.14	1.11^{b}	0.19	0.44^{c}	0.09
REG3A	1^{a}	0.24	0.05^{c}	0.01	0.19^{b}	0.01	NA	
pBD-1	1^{d}	0.11	9.75^{b}	1.38	13.49^{a}	2.74	3.48^{c}	0.48
pBD-2	1^{c}	0.17	1.11^{c}	0.03	2.02^{b}	0.33	6.30^{a}	0.42
ALP	1^{c}	0.24	1.53^{b}	0.33	3.08^{a}	0.36	1.82^{b}	0.20
HSP70	1^{a}	0.24	0.88^{b}	0.28	0.62^{b}	0.16	0.22^{d}	0.05
AMPK	1^{b}	0.19	0.24^{c}	0.05	1.64^{a}	0.39	0.30^{c}	0.06
PGC-1α	1^{c}	0.09	1.51^{a}	0.19	1.08^{bc}	0.18	1.21^{b}	0.16
Sirt1	1^{a}	0.15	0.07^{b}	0.01	0.16^{b}	0.03	0.08^{b}	0.01
UCP2	1^{c}	0.24	1.23^{c}	0.26	9.41^{a}	1.27	2.40^{b}	0.31
COX7A1	1^{b}	0.14	1.21^{a}	0.23	1.01^{b}	0.14	0.87^{b}	0.08
COX7C	1^{a}	0.11	1.09^{a}	0.08	0.82^{b}	0.14	1.15^{a}	0.08

Data are means±SD, n=8. ^{a,b}Values within a row with different letters differ (P<0.05). NA, no amplification signal was detected

The intestinal ALP is related to enterocyte proliferation and differentiation and is regarded as a key biomarker enzyme for changes in the primary digestive and absorptive functions of the small intestine (52). We found that mRNA levels for ALP in the jejunum and ileum were decreased in LPS-challenged piglets, compared with the control group (Table 3). Conversely, elevated ALP expression occurs in the small intestine of pigs (53) and rats (54) which exhibits increases in intestinal cell proliferation and absorptive function (55,56).

Additionally, many molecules play roles in transporting luminal nutrients and ions into enterocytes. The SGLT-1 is the major route for the transport of dietary sugars from the lumen into enterocytes (57), whereas the Na^{+}/K^{+}-ATPase is responsible for transporting Na^{+} into the intestinal cells in exchange for the efflux of K^{+} (58). Furthermore, CFTR is located on the apical membrane of epithelial cells and is responsible for the transport of chloride and bicarbonate ions into and out of the cell (59,60). The small intestine of piglets absorbs lesser amounts of Na^{+}, water, and glucose in the ileum at 2 h after LPS administration (61). Similarly, other animals (rabbits and rats) challenged with LPS also exhibited decreases in the jejunal absorption of solutes and the activity of Na^{+}-K^{+}-ATPase (62,63). We found

Table 3. Altered gene expression in the four intestinal segments of LPS-challenged piglets relative to unchallenged controls

Items	Duodenum fold-change[a]	Jejunum fold-change[a]	Ileum fold-change[a]	Colon fold-change[a]
EGF	0.18**	0.62*	1.32	0.70*
IGF-1	0.76	0.72*	0.65*	0.68*
mTOR	0.86	0.54*	0.62*	0.84
Ki-67	0.91	0.86	0.74*	0.90
Occludin	0.67*	0.41*	0.78*	0.93
Claudin-1	0.64*	0.74*	0.69*	0.48*
AQP-8	0.22**	0.34**	0.29**	0.48**
pBD-1	2.43**	3.65**	2.19**	3.01**
REG3A	0.41**	0.07**	0.33**	NA
SGLT-1	1.14	0.83	0.70*	1.15
CFTR	1.01	0.80	0.70*	1.42*
Na^+/K^+-ATPase	1.12	0.69*	0.93	1.06
ALP	1.01	0.73*	0.65*	1.04
HSP70	2.53**	3.18**	2.69**	2.60**
AMPK	1.28*	1.33*	1.12	1.31*
Sirt1	0.91	0.79*	0.79*	0.93
UCP2	0.56*	0.39**	0.43**	0.66*
COX7A1	0.89	0.54*	0.87	1.41
COX7C	0.64*	0.48**	1.06	1.04

The mRNA level for each gene in the control group (without LPS treatment) was set to 1.00. Only genes with statistically significant changes are shown. The values are means for 8 piglets per treatment group. [a]Differences were analyzed using the student's unpaired *t*-test. *P<0.05, **P<0.01. NA, no amplification signal was detected

that ileal SGLT-1 and CFTR, as well as jejunal Na^+ K^+-ATPase were down-regulated after LPS challenge (Table 3), indicating the dysfunction of small intestinal absorption. Notably, Groschwitz and Hogan have shown that IL-4 regulated ion conductance through down-regulation of expression of the epithelial cystic fibrosis transmembrane conductance regulator Cl^- channel (1). Interestingly, the transcript levels of intestinal amino acid or peptide transporters, such as $b^{0,+}$AT and peptide transporter (PepT1), were also decreased in response to LPS (Table 4), highlighting the intestinal dysfunction induced by LPS.

3.5. LPS regulates the expression of genes associated with intestinal mucosal energy metabolism

Regarding mucosal energy metabolism, the expression of the AMPK gene in the duodenum, jejunum and colon was up-regulated after LPS (Table 3). Since AMPK serves as a sensor of cellular energy status (64), increased expression of AMPK may help to meet an increased need for energy to protect the intestinal mucosa against the LPS-induced injury. This view is supported by our additional data on intestinal expression of regulators of cellular mitochondrial biogenesis and ATP biosynthesis. Specifically, both PGC-1α and Sirt 1 stimulate mitochondrial biogenesis in response to the cold environment or low energy supply (65,66). LPS injection may affect mitochondrial biogenesis through down-regulating expression of the Sirt 1 gene. This notion is consistent with the observation that abundance of jejunal and ileal mucosal Sirt 1 mRNA was reduced in LPS-challenged piglets. Shen *et al*. (67) have also demonstrated reductions of both mRNA and protein abundances of Sirt 1 in LPS-treated macrophages. In addition, UCP2 and COX play a critical role in cellular ATP production. UCP2 is expressed in many tissues and its mRNA has been detected in enterocyte (68). COX is the terminal oxidase of the respiratory chain and uses electrons to reduce oxygen to form (69). mRNA levels for UCP2 in the duodenum, jejunum, ileum and colon, as well as COX7A1 and COX7C in the jejunum were down-regulated by LPS challenge (Table 3), suggesting a transcriptional regulation of

Table 4. Altered gene expression in the two intestinal segments of LPS-challenged piglets relative to unchallenged controls

Items	Jejunum fold-change[a]	Ileum fold-change[a]
Immune response		
TLR4	1.51*	1.17
NF-κB	1.28*	1.00
IL-1β	1.72*	1.96*
CXCL9	2.94**	1.73*
CXCL10	3.69**	1.96*
CXCL11	5.78**	2.72**
Antivirus function		
IFN-α	1.07	1.01
IFN-β	1.03	0.82
OASL	2.24*	0.53*
OAS1	1.09	0.64*
OAS2	1.85	0.69*
MX1	3.34**	0.64
MX2	1.32*	0.60*
IFIT1	1.26	0.41**
IFIT2	1.30*	1.27
IFIT3	1.55*	0.76
IFIT5	1.61*	0.98
PKR	1.33*	1.09
Amino acids transporters		
$b^{0,+}$AT	0.68*	0.88
ASCT2	2.00*	1.56
SNAT2	1.25	1.12
PepT1	0.80*	0.83*

The mRNA level for each gene in the control group (without LPS treatment) was set to 1.00. The values are means for 8 piglets per treatment group. [a]Differences were analyzed using the student's unpaired *t*-test. *P<0.05, **P<0.01

these genes in association with intestinal energy metabolism. Similarly, Kizaki *et al*. (70) demonstrated a reduction in the expression of UCP2 mRNA and protein in LPS-stimulated macrophages. Furthermore, LPS depresses the activity of COX and changes the stability of transcripts for the COX subunit I (71,72). Therefore, the reduced abundance of COX7A1 and COX7C mRNA may result from a LPS-induced instability of the transcripts for COX7A1 and COX7C.

3.6. LPS regulates the expression of genes associated with intestinal mucosal immune response

An intraperitoneal LPS challenge is sufficient to induce intestinal inflammation (3). Hou *et al*. (14) reported that the levels of pro-inflammatory mediators (i.e., IL-6, TNF-α, cortisol, and PGE_2) were significantly increased in the small intestine of LPS-challenged piglets. Similarly, LPS administration increased the mRNA levels for IL-1β, chemokines (CXCL9, CXCL10, and CXCL11), TLR4, and NF-κB in the jejunum (Table 4). As a main component of the outer membrane of Gram negative bacteria, LPS directly activates the host toll-like receptor 4 (TLR4), which subsequently transduces immune-related signals to the nucleus via transcription factors, including NF-κB (73), leading to the transcription of various pro-inflammatory cytokines (74). Therefore, the TLR4-NF-κB signaling pathway may be the main mechanism involved in the immune response to LPS in porcine intestine. Inhibition of the TLR4-NF-κB signaling pathway is likely an effective strategy for treating inflammatory bowl diseases. In support of this view, glutamine inhibits TLR4 signaling in LPS-treated porcine enterocytes (75).

3.7. LPS regulates the expression of genes associated with intestinal antivirus function

The cellular response to viral infection includes the induction of genes for type I interferons, IFN-α and IFN-β, which are produced in most cell types and play a vital role in innate resistance to viral and bacterial infections (76,77). IFN-α/β can induce the expression of genes encoding for antiviral proteins, such as myxovirus (MX), 2'-5' oligoadenylate synthetases (OAS), protein kinase R (PKR), and IFN-induced protein with tetratricopeptide repeats (IFITs) (78). LPS is well known to enhance the expression of type I interferons in macrophages, particularly of IFN-β (79), suggesting that antiviral gene expression may be enhanced in response to bacterial infection. Here we found that LPS administration increased the jejunal mRNA levels for antiviral genes, such as MX1, OASL, PKR, and IFITs, but had no effect on those for IFN-α and IFN-β (Table 4). Similarly, LPS treatment did not increase expression of the IFN-α/β gene or interferon-stimulated genes, but augmented MX1 expression (76). These results suggest that secretion of mediators, including IFN-α/β or signal transducers and activators of transcription (STATs), is not involved in LPS-induced antiviral genes expression. Doyle et al. (80) suggested that selective activation of the TLR3/TLR4-interferon regulator factor 3 (IRF3) pathway potently inhibited viral replication. Likewise, Malcolm and Worthen (76) found that the signal transduction pathways necessary for induction of antiviral genes (e.g., p38), was activated by a TLR4 ligand, rather a TLR2 ligand. Therefore, LPS may induce the expression of antiviral genes via the TLR4/p38 pathway independently of type I interferons.

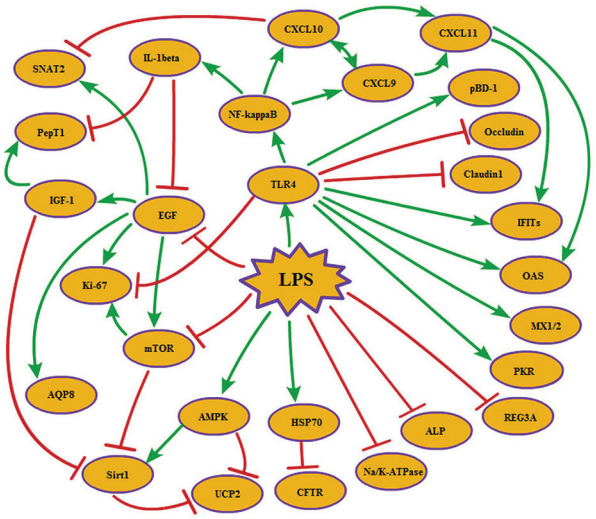

Figure 2. Proposed network for the regulation of gene expression in the jejunum of LPS-challenged piglets (based on the pathway analysis using the Pathway Studio® software).

3.8. Gene networks regulated by LPS

Based on the analysis of tested genes in the jejunum of LPS-challenged piglets, gene networks regulated by LPS are proposed in Figure 2. As indicated in Figure 2, LPS up-regulated the expression of genes involved in TLR4 and AMPK signaling, but down-regulated the expression of genes involved in EGF and mTOR signaling. Specifically, LPS increased the mRNA levels for TLR4, NF-κB, IL-1 beta, CXCL9, and CXCL10, substantiating the notion that the TLR4-NF-κB signaling pathway may be the main mechanism involved in the immune response to LPS in the porcine intestine. Though the IFN-α/β mRNA abundance was not affected by LPS, the mRNA levels for the antiviral genes, such as IFITs, OAS, MX, and PKR, were up-regulated, indicating an alternative mechanism whereby LPS regulated intestinal antiviral gene expression. Additionally, LPS down-regulated the expression of EGF and mTOR, and both of which were involved in intestinal mucosal growth and recovery (5,14). It is possible that LPS inhibits expression of EGF, whereby reducing mRNA levels for Ki-67, peptide transporter 1 (PepT1), AQP8, and sodium-coupled neutral amino acid transporter 2 (SNAT2). Similarly, there was a negative cross-talk between mTOR and AMPK (16). It remains to be determined whether LPS up-regulates AMPK expression via decreasing mTOR activity. Finally, reduced expression of REG3A, ALP, Na^+/K^+-ATPase, occludin, and claudin1 may contribute to intestinal dysfunction.

4. CONCLUSION AND PERSECTIVES

In pigs, over 85% of genes are co-expressed along the small and large intestines with regional differences in gene expression patterns. Using the model of intraperitoneal LPS-challenged piglets, we have characterized differential expression of genes associated with intestinal growth, absorption, mucosal barrier function, and energy metabolism in four intestinal segments. The duodenum, jejunum, ileum and colon respond to LPS treatment in a similar pattern. Additionally, the jejunum and ileum exhibit greater responses to LPS challenge than the duodenum and colon with regard to gene expression. Furthermore, LPS regulates mRNA levels for intestinal antiviral genes via the TLR4 pathway independently of type I interferons. These findings have important implications for understanding molecular mechanisms responsible for LPS-induced intestinal injury and dysfunction.

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Abbreviations: ALP: alkaline phosphatase; AMPK: AMP-activated protein kinase; AQP8: Aquaporins 8; AR: amphiregulin; CFTR: cystic fibrosis transmembrane conductance regulator; COX: cytochrome c oxidase; EGF: epidermal growth factor; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; IFIT, IFN-induced protein with

tetratricopeptide repeat; IFN, interferon; IGF-1: insulin-like growth factor 1; IGFBP-3: insulin-like growth factor binding protein 3; mTOR: mammalian target of rapamycin; MX, myxovirus; OAS, 2'-5' oligoadenylate synthetases; PepT1, peptide transporter 1, pBD-1: porcine β-defensins 1; PGC-1α: peroxisome proliferator-activated receptor-γ coactivator 1α; Sirt1: sirtuin 1; REG3A: regenerating islet-derived protein 3 alpha; RPL4: Ribosomal Protein L4; SGLT-1: Sodium/Glucose co-transporter 1; SNAT2, sodium-coupled neutral amino acid transporter 2; UCP-2: uncoupling protein 2; ZO-1: zonula occluden 1.

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