The roles of the TLR/NF-κB signaling pathway in the mutual interactions between the lung and the large intestine

WEN FU, JING ZHAO, XINGLONG LIU, YONGXIANG GAO and CHUAN ZHENG

Basic Medical College, Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan 610075, P.R. China

Received October 25, 2017; Accepted May 1, 2018

DOI: 10.3892/mmr.2018.9111

Abstract. The 'exterior-interior relationship between the lung and the large intestine' is a classical basic theory in Traditional Chinese Medicine. The present study aimed to investigate the roles of the toll like receptor/nuclear factor-κB (TLR/NF-κB) signaling pathway in the mutual interactions between the lung and the large intestine. A rat model of allergic asthma complicated with intestinal flora disorder was established by oral administration of *Candida albicans* and intraperitoneal injection with ovalbumin. The number of inflammatory cells and expression levels immunoglobulin (Ig) E, secretory IgA, interleukin (IL)-4 and interferon-γ in serum and bronchoalveolar lavage fluid were subsequently measured. Bacterial colonies and expression of 16S ribosomal DNA were studied in feces samples and pathological alterations of lung tissues were identified. Furthermore, the expression levels of genes associated with the TLR/NF-κB signaling pathway in the lung and intestinal tissues were determined by reverse transcription-quantitative polymerase chain reaction. The results of the present study indicated that, in the rat model of allergic asthma complicated with intestinal flora disorder, the expression levels of IL-4 and IgE, and the numbers of inflammatory cells and *C. albicans* increased, and marked inflammatory cell infiltration was observed in lung tissues, suggesting that the animal model was successfully established. Furthermore, the present results revealed the mRNA expression levels of genes associated with the TLR/NF-κB signaling (including myeloid differentiation primary response 88, TNF receptor associated factor 6 and β-arrestin) were upregulated in both of the lung and intestinal tissues of the model group rats. Collectively, the results demonstrated that the TLR/NF-κB signaling may serve roles in the mutual interactions between the lung and the large intestine, and TLR and NF-κB may be potential targets for the treatment of lung diseases complicated with intestinal disorders.

Correspondence to: Professor Yongxiang Gao or Professor Chuan Zheng, Basic Medical College, Chengdu University of Traditional Chinese Medicine, 1166 Liutai Avenue, Chengdu, Sichuan 610075, P.R. China
E-mail: gaoyxcdtcm@126.com
E-mail: zhengchuancdutcm@126.com

Key words: toll like receptor/nuclear factor-κB, allergic asthma, intestinal flora disorder, exterior-interior relationship between the lung and the large intestine, mutual interactions

Introduction

In Traditional Chinese Medicine, the 'exterior-interior relationship between the lung and the large intestine' is a classical basic theory, which was first postulated in the Inner Canon of Huangdi ancient Chinese medical text (1,2). This theory serves as guidance for treatment of certain pulmonary diseases combined with disorders of the large intestine (3). In addition, an increasing amount of clinical evidence demonstrated that there is a relationship between the physiology and pathology of the lung and large intestine (4,5). However, at present, systemic reports regarding molecular mechanisms underlying mutual interactions between the lung and the large intestine are lacking.

Allergic asthma is a complex chronic airway inflammatory reaction mediated by mastocytes, eosinophils and T lymphocytes (6,7). The prevalence of asthma in industrialized countries has been increasing and asthma is now the most common chronic disease of children in the United States (8). It is believed that an additional 100 million people will be suffering with asthma by 2025 (9). Therefore, allergic asthma has become a public health issue. Increasing amount of research indicated that allergic asthma is closely associated with the intestinal flora disorder and is considered a typical disease model for investigating the mutual interactions between the lung and the large intestine (1,10). Therefore, in the present study, an animal model of allergic asthma complicated with intestinal flora disorder was established in rats to elucidate the molecular mechanism of mutual interactions between the lung and the large intestine.

Materials and methods

Animals. A total of 30 male Sprague-Dawley rats (3-4 weeks old, 200±20 g) were purchased from the Dashuo Laboratory Animal Co., Ltd. (Chengdu, China; http://www.jianyang.ccoo.cn/post/zhaopin/minqi/index462587.html). The animals were housed in a temperature and humidity controlled room (temperature 22±2˚C, atmosphere 40-60% CO_2, and 10-12-h light/dark cycle) with food and water *ad libitum*. All animal experimental protocols were approved by the Ethics

Committee for Laboratory Animal Experimentation of Chengdu University of Traditional Chinese Medicine.

Chemicals and reagents. Cefoperazone was purchased from the North China Pharmaceutical Co., Ltd. (Shijiazhuang, China). Culture media (the selective culture media for *Enterococcus*, the selective culture media for enteric Bacilli, the *BS* culture media for *Bifidobacterium*, the selective culture media for *Lactobacillus* and the *SDA* culture media for *Candida*) for *Enterococcus*, enteric Bacilli, *Bifidobacterium*, *Lactobacillus* and *Candida* were purchased from the Qingdao Haibo Biotechnology Co., Ltd. (Qingdao, China). Ovalbumin (OVA) was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Aluminum hydroxide was purchased from Chengdu Chron Chemicals Co., Ltd., (Chengdu, China). Live combined *Bacillus subtilis* and *Enterococcus faecium* granules (BEG) were purchased from the Hanmi Pharmaceutical Co., Ltd. (Beijing, China). Aminophylline (ANP) was purchased from the Southwest Pharmaceutical Co., Ltd. (Chongqing, China). *Candida albicans* was purchased from the Guangdong Huankai Microbial Technology Co., Ltd. (Guangzhou, China; http://huankaiye.bioon.com.cn/). TRIzol reagent was purchased from Invitrogen (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Rat ELISA kits for secretory(s) immunoglobulin (Ig) A (cat. no. 201411), IgE (cat. no. R141126-117a) interleukin (IL)-4 (cat. no. R141126-002a) and interferon (IFN)-γ (cat. no. R141126-101a) were purchased from the Neobioscience Technology Co., Ltd. (Shenzhen, China). HiScript 1st Strand cDNA Synthesis kit and SYBR-Green Master Mix were purchased from the Vazyme Biotech Co., Ltd. (Nanjing, China). Hematoxylin and eosin (H&E) and Wright-Giemsa kits were purchased from the Baso Biotech Co., Ltd. (Taiwan, China). All primers used in the present study were designed by Primer-Express version 3.0 (Thermo Fisher Scientific, Inc.) and synthetized by Sangon Biotech Co., Ltd. (Shanghai, China; Table I).

Experimental protocols and animal model preparation. A total of 30 rats were randomly divided into 3 groups (n=10): i) Normal group; ii) model group; and iii) positive treatment group (treated with live combined BEG and ANP). With the exception of the normal rats, all animals in the model and positive groups were freely administered orally Cefoperazone aqueous solution (0.5 g/l) for consecutive 5 days. Subsequently, rats of the model and positive treatment groups were orally administered 50 μl *Candida albicans* [10^9 colony forming unit (CFU)/ml] on the sixth day. OVA was used to induce allergic asthma in rats according to the previous methods (11,12) with minor modifications. Briefly, rats were immunized via intraperitoneal (IP) injection of 1 ml OVA-aluminum hydroxide mixture on days 0 and 7 (1 mg OVA and 200 mg aluminum hydroxide were dissolved in 1 ml saline). Subsequently, allergic asthma in rats was induced with 1% OVA-saline solution by aerosol inhalation in a glass box (10x10x20 cm) from day 14 to day 21 (30 min/day). Rats in the normal group were treated using the same protocol, with saline instead of OVA-aluminum hydroxide mixture. In the positive treatment group, rats were orally administered BEG (500 mg/kg) and ANP (300 mg/kg) from day 14 to day 21. On the day 21, the pulmonary functions were determined, then rats were sacrificed under pentobarbital sodium anesthesia (45 mg/kg; IP) after the blood samples and bronchoalveolar lavage fluid (BALF) were collected. Subsequently, the lung tissues, the large intestine tissues and intestinal mucous were harvested for the following biochemical assays.

Determination of the rat pulmonary function. Rat pulmonary function including respiratory rate and airway responsiveness were determined using a Buxco® animal pulmonary function analysis system (FinePointe Non-Invasive Airway Mechanics; DSI; Harvard Bioscience, Inc., Holliston, MA, USA; https://www.datasci.com/products/buxco-respiratory-products/finepointe-non-invasive-airway-mechanics). Airway responsiveness was evaluated using the enhanced pause values (Penh value) according to a previously reported method (13).

Blood cell counting. Blood smears were prepared with blood taken from the heart of the rats and fixed with formalin for 2-3 min at 25˚C. Subsequently, Wright-Giemsa staining was performed for 15 min at 25˚C and cell counting was carried out under an optical light microscope (CH20BIMF200; Olympus Corporation, Tokyo, Japan) at magnification, x100 and x400.

Bacterial colony counting. All experiments were carried out under sterile conditions. Briefly, 0.1 g rat feces were dissolved in saline at a dilution of 1:10^{10}. Subsequently, the diluted feces samples were cultured in an anaerobic incubator for 48-72 h at 37˚C for detection of *Bifidobacterium*, *Lactobacillus* and *Candida albicans*. Diluted feces samples were also cultured in an aerobic incubator for 24-48 h at 37˚C for detection of *Enterococcus* and *Enterobacterium*. The colony counting was expressed as logCFU/g.

Examination of pathological alterations of the lung tissues. The histopathological examination was performed as previously described (14). Briefly, the lung tissues were collected and fixed with 4% paraformaldehyde for 24 h. The tissues were subsequently embedded in paraffin and cut into 5-μm-thick sections. The samples were deparaffinized and stained with H&E. Finally, pathological alterations of lung tissues were examined under an optical microscope (CH20BIMF200; Olympus Corporation).

ELISA assays for detection of sIgA, IgE, IL-4 and IFN-γ. The levels of sIgA in BALF and intestinal mucosa, and levels of IgE, IL-4 and IFN-γ in serum were determined using commercial ELISA kits according to the manufacturers' protocols and determined using a microplate reader at a wavelength of 450 nm (Multiskan Ascent 413MBY042078; Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays. Lung and intestine tissues were collected and homogenized. Total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Subsequently, total RNA was used for cDNA synthesis using HiScript 1st Strand cDNA Synthesis kit, according to the manufacturer's protocol. qPCR was performed using SYBR-Green Master Mix, according to the manufacturer's protocol with primers specific to TRL-2, TRL-4, myeloid differentiation primary response 88

Table I. Primers used in this research.

	Primer sequence (5'→3')	
Gene	Forward	Reverse
TLR-2	5'-GTTGCGTTACATCTTGGA-3'	5'-GGAATACACAGTGCTCAG-3'
TLR-4	5'-CAGCTCGTTTCTCACCCAGT-3'	5'-TGTATCGGTGGTCAGTGTGC-3'
MyD88	5'-CGACGCCTTCATCTGCTA-3'	5'-GCCGATAGTCTGTCTGTTCT-3'
TRAF6	5'-CAGTCCCCTGCACATTCAGT-3'	5'-CTGGGCCAACAGTCTCATGT-3'
β-arrestin	5'-GGGCATTTGTACTGAGCTGT-3'	5'-TGCACCTTGAGGCATCTCTG-3'
β-actin	5'-AGGGAAATCGTGCGTGACAT-3'	5'-GAACCGCTCATTGCCGATAG-3'

TLR, toll like receptor; MyD88, myeloid differentiation primary response 88; TRAF6, TNF receptor associated factor 6.

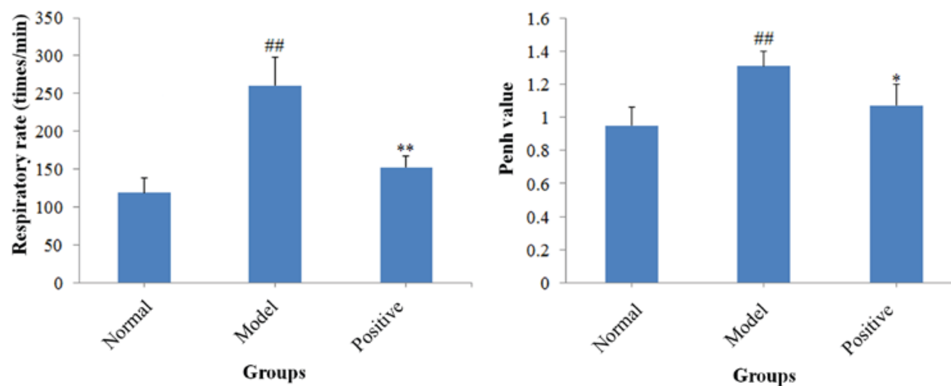

Figure 1. Pulmonary function determined by measuring the respiratory rate and Penh value. Data are presented as the mean ± standard deviation (n=10). *P<0.05 and **P<0.01 vs. the model rats; ##P<0.01 vs. the normal rats.

(MyD88), TRAF6, β-arrestin and β-actin (Table I). The thermocycling conditions for qPCR: Pre-denaturation at 95˚C for 5 min and 95˚C for 10 sec at 53.5˚C for 30 sec, the data were recorded by fluorescent reading board, and 39 cycles were recorded. The PCR reactions were performed using CFX96TM™ Real-Time system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The relative mRNA expressions were determined by $2^{-\Delta\Delta Cq}$ relative quantitative analysis according to the previous reported method (15).

Rat intestine 16S ribosomal DNA (16S rDNA) assay. For the determination of rat intestine 16S rDNA, 6 rats were selected from each group (n=6). Subsequently, the DNA was collected from the feces of rats. The DNA samples used in this study were isolated and purified using the QIAamp® DNA Stool isolation and purification kit (Qiagen China Co., Ltd., Shanghai, China) and the protocol is adopted following the manufacturer's protocol. Further analysis was performed using a DNA sequencer (Illumina HiSeq™ 2000; Illumina, Inc., San Diego, CA, USA) and BGI Tech Solutions Co., Ltd., (Shenzhen, China) analyzed and interpreted the sequencing data and the parameters were investigated as described previously (16,17).

Statistical analysis. Data are presented as the mean ± standard deviation, and each experiment was repeated at least 3 times. Differences between groups were determined using one-way analysis of variance followed by Dunnett's multiple comparisons test. The statistical significance of differences was analyzed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Results of the determination of pulmonary function. The respiratory rate and Penh value were determined (Fig. 1). Compared with the normal rats, the respiratory rate (P<0.01) and Penh value (P<0.01) of the model rats increased significantly. However, the positive drug treatment significantly decreased the elevated respiratory rate (P<0.01) and Penh value (P<0.05) compared with the model rats. The present results revealed that a rat model of allergic asthma was successfully established.

Results of cell counting in blood samples. The results of cell counting (Table II) indicated that the numbers of the four types of inflammatory cells (eosinophils, neutrophils, lymphocytes and monocytes) increased in the model rats compared with the normal rats (all P<0.01). By contrast, the positive treatment decreased the number of inflammatory cells compared with the model group (P<0.01, P<0.05, P<0.05 and P<0.01, respectively for eosinophils, neutrophils, lymphocytes and monocytes).

Results of bacterial colony counting in rat feces. The number of colonies of three bacterial strains including *Enterococcus*,

Table II. Blood cell count (x10^6/l).

Group	Inflammatory cell type			
	Eosinophil	Neutrophil	Lymphocyte	Monocyte
Normal	2.66±1.2	22.50±5.5	37.00±5.0	2.00±0.36
Model	27.75±2.19^c	43.00±1.82^c	66.80±3.21^c	5.33±0.88^c
Positive	4.66±0.55^b	32.00±2.08^a	50.40±4.93^a	2.88±0.26^b

Data are presented as the mean ± standard deviation (n=10). ^{a}P<0.05, ^{b}P<0.01, vs. model rats; ^{c}P<0.01, vs. normal rats.

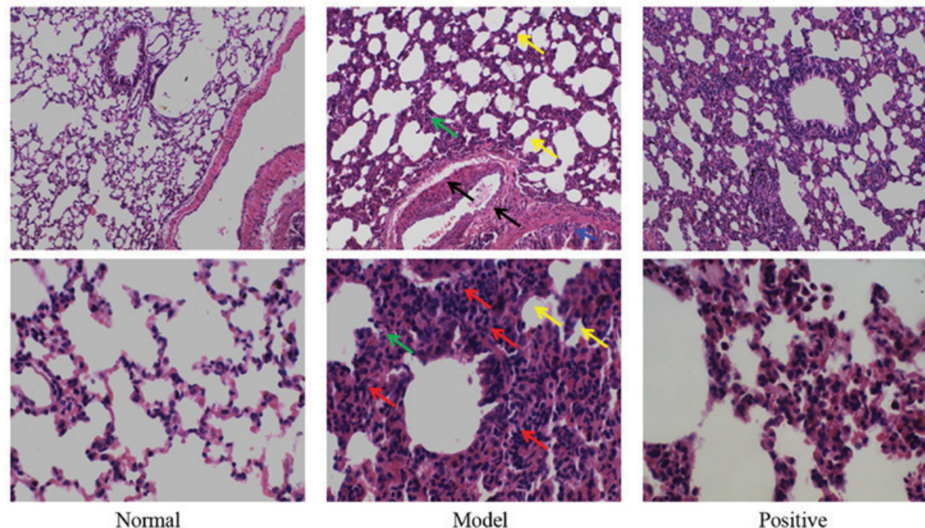

Figure 2. Histological examination of lung tissues with hematoxylin and eosin staining (top row magnification, x100; bottom row magnification, x400). Black arrows indicate the blood vessel; red arrows indicate the inflammatory cell infiltration; yellow arrows indicate the pulmonary alveol; green arrows indicate the pulmonary septum; blue arrows indicate the bronchus.

Bifidobacterium and *Lactobacillus* significantly decreased in model rats compared with normal rats (all P<0.01; Table III), whereas the numbers of colonies of enteric Bacilli (P<0.05) and *Candida albicans* (P<0.01) significantly increased. By contrast, in the positive drug treatment group, the number of *Enterococcus* (P<0.01), *Bifidobacterium* (P<0.01), enteric Bacilli (P<0.05) and *Lactobacillus* (P<0.01) colonies significantly increased, whereas the colony numbers of *Candida albicans* decreased (P<0.01), compared with the model rats. The above results indicated that the model rats exhibited significant alterations of the intestinal flora.

Examination of pathological alterations of the lungs. Results of the pathological examination indicated that, in the normal group, no obvious pathological alterations were observed (Fig. 2). Compared with the normal rats, marked inflammatory cell infiltration was observed in the lung tissues of the model rats, and the pulmonary septum became thick and alveolar space became narrow (Fig. 2). In addition, edema could be also observed in the blood vessel and tracheal wall. However, in the positive group, the aforementioned abnormal alterations were markedly alleviated.

Results of the ELISA assays. sIgA levels in both BALF (P<0.05; Fig. 3A) and intestinal mucosa (P<0.01; Fig. 3B) decreased in the model rats compared with the normal rats, and the serum IgE levels significantly increased (P<0.01; Fig. 3C). Following treatment with BEG and ANP, the sIgA levels in the intestinal mucosa (P<0.05) increased, whereas the serum IgE levels decreased (P<0.01), compared with the model rats.

Following activation with OVA, the IL-4 levels (P<0.01) of the model rats significantly increased compared with the normal group, whereas the IFN-γ levels significantly decreased (P<0.05; Fig. 4). By contrast, in the positive treatment group, the levels of IL-4 significantly decreased (P<0.01; Fig. 4A) and the levels of IFN-γ (P<0.05; Fig. 4B) significantly increased compared with the model rats.

Results of the 16S rDNA assays of the rat intestine. Following discarding the low-quality sequencing reads using an inner program (BGI Tech Solutions, Co., Ltd.) to generate clean data, a detailed result for each sample was obtained. The operational taxonomic units (OTUs) analysis revealed the OUT numbers of the model rats were lower than that of normal and positive-treated rats, indicating the species richness of the model rats was lower than that of the normal and positive-treated rats (Table IV). Furthermore, the number of bacteria constituting normal intestinal flora (including Bacteroidetes and *Prevotella*) decreased compared with the normal and positive groups (Fig. 5). By contrast, the number

Table III. Intestinal flora determination (log colony forming unit/g).

Group	Gut microorganism				
	Enterococcus	Enteric bacilli	*Bifidobacterium*	*Lactobacillus*	*Candida albicans*
Normal	6.88±0.67	6.78±0.69	10.66±1.63	9.28±0.64	0.00±000
Model	4.70±0.46[c]	7.99±0.96[b]	0.00±0.00[c]	0.00±0.00[c]	7.30±0.50[c]
Positive	5.78±0.69[a]	6.78±0.67	1.68±0.59[a]	8.86±0.55[a]	3.04±0.59[a]

Data are presented as the mean ± standard deviation (n=10). [a]P<0.01, vs. the model rats; [b]P<0.05, [c]P<0.01 vs. normal rats.

Table IV. Results of the OTU analysis of samples.

Group	Tag number	OTU number	OTU number (removing singletons)
Normal	14.751	828	369
Control	18.346	593	346
Positive	16.844	718	385

OTU, operational taxonomic unit.

of Butyricimonas increased in the model group compared with the normal and positive rats. These results demonstrated that intestinal flora disorder was observed in the model rats. All of the above results indicated that a rat model of allergic asthma complicated with intestinal flora disorder was successfully established, and was subsequently used for the further investigation of the underlying molecular mechanisms.

Results of the RT-qPCR assays. Following successful establishment of the rat model of allergic asthma complicated with intestinal flora disorder, the potential molecular mechanisms of mutual interactions between the lung and the large intestine were investigated using RT-qPCR assays. The mRNA expression levels of TRL-2, TRL-4, MyD88, TRAF6 and β-arrestin were significantly upregulated in both the lung and intestinal tissues of the model rats, compared with the normal rats (all P<0.01; Fig. 6). By contrast, treatment with BEG and ANP significantly decreased the upregulated mRNA expression levels of TRL-2, TRL-4, MyD88, TRAF6 and β-arrestin (all P<0.01; Fig. 6) in both the lung and intestinal tissues, compared with the model group.

Discussion

In the present study, using a rat model of allergic asthma complicated with intestinal flora disorder, the roles of the TLR/NF-κB signaling pathway in the mutual interactions between the lung and the large intestine were studied. The results of the present investigation indicated that allergic asthma was associated with the intestinal flora disorder and the TLR/NF-κB signaling pathway may serve a role in this association.

Establishing a suitable and reliable animal model is the crucial initial step for investigating the mutual interactions between the lung and the large intestine (18,19). The present investigation successfully constructed a rat model of allergic asthma complicated with intestinal flora disorder. The results indicated that the rats in the model group exhibited obvious characteristics of allergic asthma and intestinal flora disorder, which were demonstrated by the pathological alterations, ELISA results (sIgA, IgE, IFN-γ and IL-4 levels), blood cell count and bacterial count in feces, as well as the 16S rDNA assay of the rat intestine. In the model rats, the levels of IL-4 and IgE, and the number of inflammatory cells and *Candida albicans* increased, and obvious inflammatory cell infiltration was observed in the lung tissues compared with the normal rats.

The sIgA is a marker for the intestinal flora disorder and damage of intestinal mucosa, and a previous report revealed that the number of intestinal *Bifidobacteria* is closely associated with the level of sIgA (20). In the model group the sIgA expression levels decreased in both of the intestinal mucosa and BALF, which supports the 'exterior-interior relationship between the lung and the large intestine' theory. TLRs, pattern recognition receptors expressed in the cytomembrane, are closely associated with the body immunocompetence (21). TLRs can recognize specific conserved molecular components of microorganisms and transfer the signals into the cell, leading to the activation of NF-κB (21,22). Furthermore, activation of NF-κB can induce the excessive release of pro-inflammatory cytokines including IL-1, IL-6, TNF-α and IL-12 (23). The characteristic pathological alterations associated with asthma include airway inflammatory reactions and airway remodeling (12). TLRs serve roles in the development of airway inflammatory reactions and further activate the MyD88- IRAK-TRAF6-IKK-NF-κB signaling pathway, resulting in excessive release of pro-inflammatory cytokines and inflammation (24-26). In addition, TLRs promote the maturation and differentiation of immune cells but also the transdifferentiation of CD^{4+} T cells into T regulatory cells (Tregs). Therefore, the TLR/NF-κB signaling pathway could further regulate the balance of type 1 T helper/type 2 T helper cells via Tregs (27). Recently, it has been reported that the TLR/NF-κB signaling serves roles in the intestinal defense against pathogens and maintenance of immune system homeostasis and intestinal flora balance (28). A previous study indicated that TLR-2 and TLR-4 are closely associated with the recognition of peptidoglycan and lipopolysaccharide (29), respectively. Additionally, TLR-2 and TLR-4 serve roles in the development of allergic asthma and intestinal flora-associated diseases (30,31). In addition, β-arrestin was upregulated in patients with allergic asthma and served a role in the development of chronic intestinal

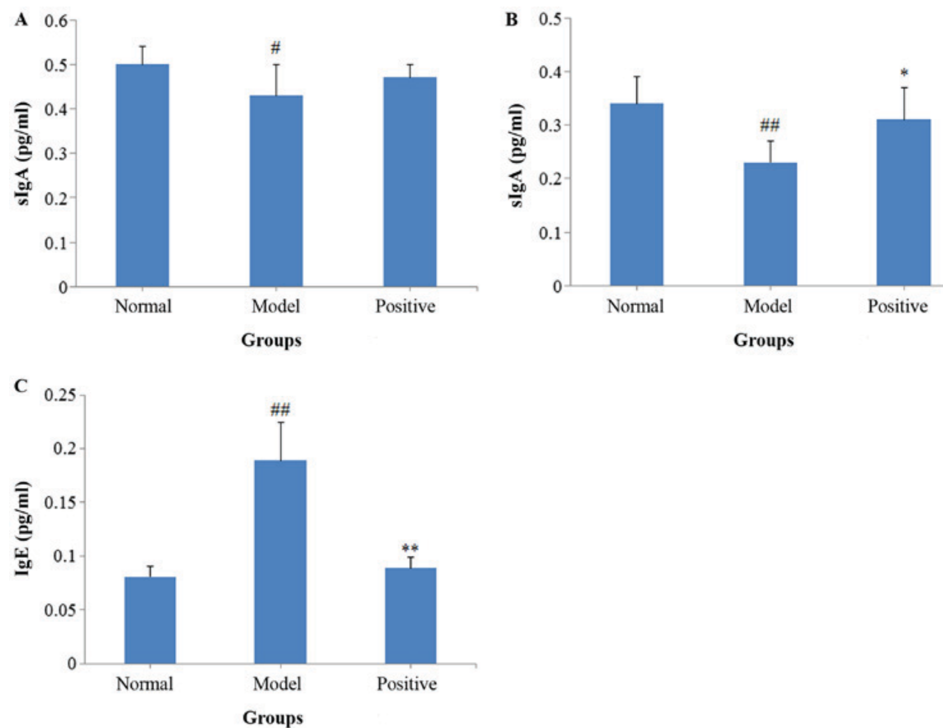

Figure 3. ELISA assays. (A) sIgA in bronchoalveolar lavage fluid. (B) sIgA in intestinal mucosa. (C) IgE in serum. Data are presented as the mean ± standard deviation (n=10); *P<0.05 and **P<0.01 vs. the model rats; #P<0.05 and ##P<0.01 vs. the normal rats. S, secretory; Ig, immunoglobulin.

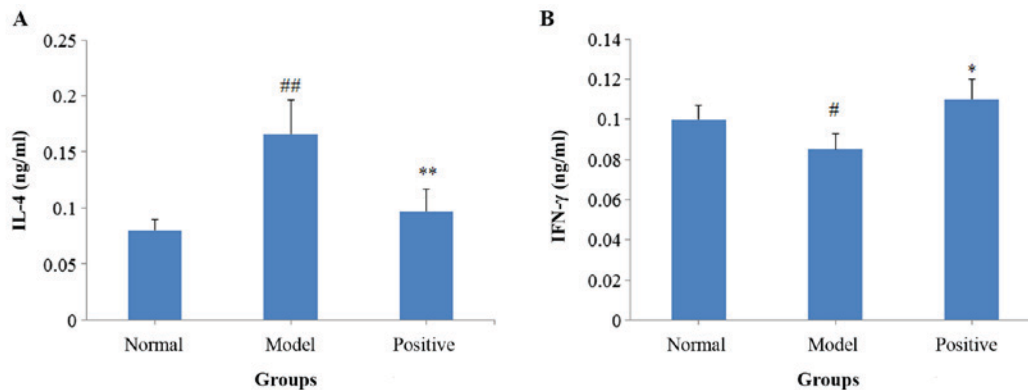

Figure 4. ELISA assays in BALF. Expression levels of (A) IL-4 and (B) IFN-γ in BALF. Data are presented as the mean ± standard deviation (n=10). *P<0.05 and **P<0.01 vs. the model rats; #P<0.05 and ##P<0.01 vs. the normal rats. BALF, bronchoalveolar lavage fluid; IL-4, interleukin 4; IFN-γ, interferon-γ.

inflammation (32-34). Therefore, β-arrestin may serve a role in the development of allergic asthma and intestinal diseases. In the present study, mRNA expression levels of TRL-2, TRL-4, MyD88, TRAF6 and β-arrestin significantly increased in both the lung and intestinal tissues of the model rats compared with the normal rats. By contrast, treatment with BEG and ANP could decrease the up-regulated mRNA expression levels of these genes in both the lung and intestinal tissues of the model rats. The results if the present study indicated that the TLR/NF-κB signaling is a potential link between asthma and intestinal disorders in the rat models and may also be a molecular mechanism of the 'exterior-interior relationship between the lung and the large intestine'. Furthermore the present study demonstrated, the upregulated TLR/NF-κB signaling is an important molecular mechanism for the development of lung diseases complicated with intestinal disorders.

'Exterior-interior relationship between the lung and the large intestine' is a classical basic theory in Traditional Chinese Medicine and the present study did not prove the direct mutual interaction between the lung and the large intestine. However, future studies should aim to investigate the direct mutual interactions between the two organs in animal models. The present study indicated that the TLR/NF-κB signaling may serve a role in the mutual interactions between the lung and the large intestine, however, alternative signal transduction mechanisms have not been investigated. Furthermore, the present study analyzed the alterations of intestinal flora by sequencing 16S rDNA, however, flora metagenomics analysis of both the lung and intestine would be an improved strategy for investigating the molecular mechanisms of the two organs the association between them.

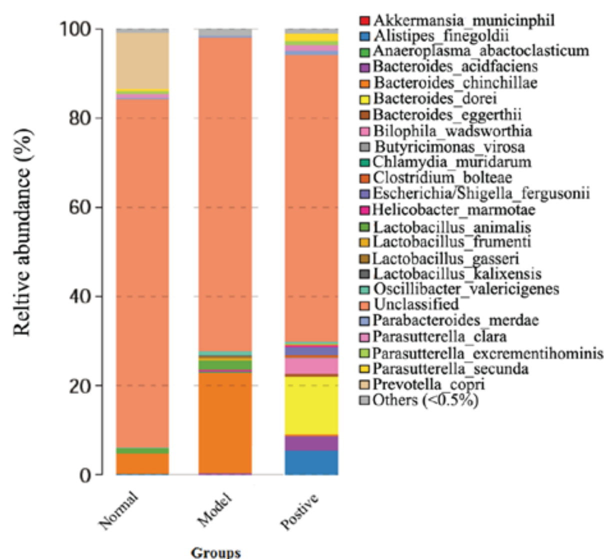

Figure 5. Composition of the intestinal flora in the normal, model and positive groups.

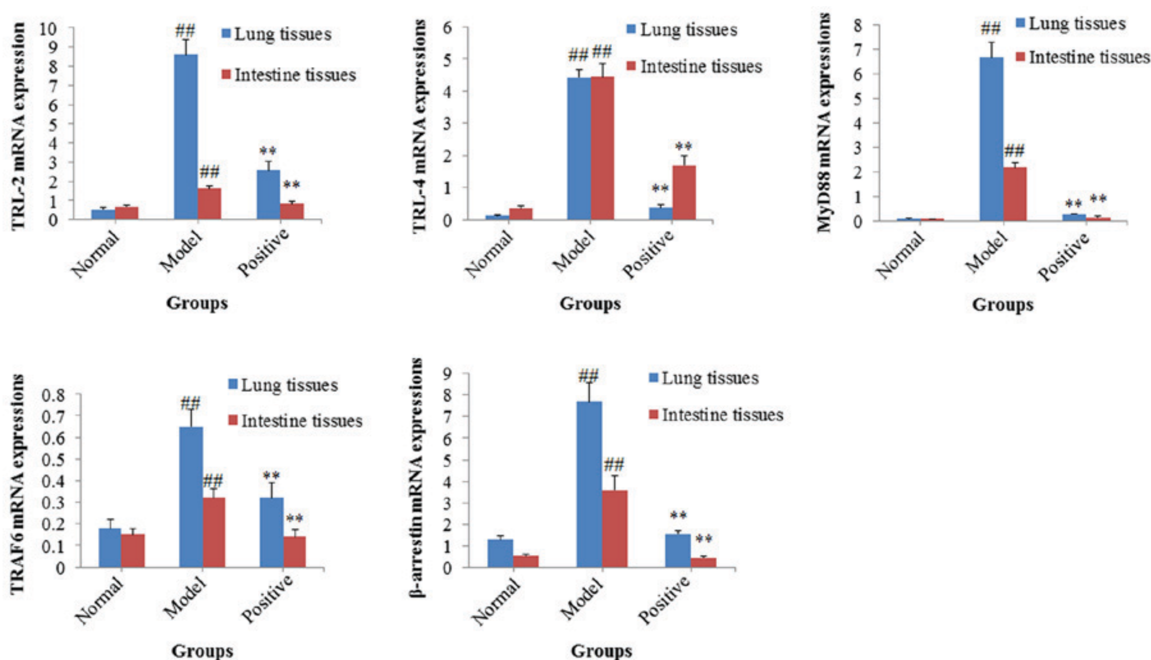

Figure 6. Results of reverse transcription-quantitative polymerase chain reaction analysis of expression levels of TLR-2, TLR-4, MyD88, TRAF6 and β-arrestin. Data are presented as the mean ± standard deviation (n=10). **P<0.01 vs. the model rats; ##P<0.01 vs. the normal rats. TLR, toll like receptor; MyD88, myeloid differentiation primary response 88; TRAF6, TNF receptor associated factor 6.

In conclusion, the present experimental results revealed that the TLR/NF-κB signaling may serve roles in the mutual interactions between the lung and the large intestine. The results also support the Traditional Chinese Medicine theory of 'exterior-interior relationship between the lung and the large intestine'. Furthermore, the results of the present study suggested that the TLR/NF-κB signaling is a potential target for the clinical treatment of lung diseases complicated intestinal disorders.

Acknowledgements

Not applicable.

Funding

The authors are grateful for the financial support from the National Natural Science Foundation of China (grant no. 81303085) and the Foundation of Science and Technology Department of Sichuan Province (grant no. 2013JY0067).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YG and CZ designed the experiment; WF, JZ, XL completed the experiments and analyzed the experimental data; WF and CZ wrote the paper.

Ethics approval and consent to participate

All animal experimental protocols were approved by the Ethics Committee for Laboratory Animal Experimentation of Chengdu University of Traditional Chinese Medicine.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Yin LM, Zhang GQ, Yan XK, Wang Y, Xu YD and Yang YQ: An in vivo and in vitro evaluation of the mutual interactions between the lung and the large intestine. Evid Based Complement Alternat Med 2013: 695641, 2013.
2. Ding H: Huang Di Nei Jing Ling Shu. Sichuan: Si Chuan Science and Technology Publishing house: 27-28, 2008.
3. Yan XK, Wang Y, Zhang GQ, Yang YQ and Cui LP: The research progress of exterior-interior relationship between the lung and the large intestine. Shaanxi J TCM 24: 378-380, 2003.
4. Yang F, Wang J and Wang Q: Allergic diseases and intestinal flora imbalance for allergic constitution research. J Beijing Univ Tradit Chin Med 38: 509-514, 2015.
5. Jia JJ, Chen X and Jie JP: Modern research of the exterior-interior relationship between the lung and the large intestine. Acta Chin Med Pharmacol 34: 23-25, 2011.
6. Lee MY, Shin IS, Jeon WY, Lim HS, Kim JH and Ha H: Pinellia ternata Breitenbach attenuates ovalbumin-induced allergic airway inflammation and mucus secretion in a murine model of asthma. Immunopharmacol Immunotoxicol 35: 410-418, 2013.
7. Yacoub MR, Colombo G, Marcucci F, Caminati M, Sensi L, Di Cara G, Frati F and Incorvaia C: Effects of sublingual immunotherapy on allergic inflammation: An update. Inflamm Aller Drug Targets 11: 285-291, 2012.
8. Nakajima Y, Goldblum RM and Midoro-Horiuti T: Fetal exposure to bisphenol A as a risk factor for the development of childhood asthma: An annimal model study. Environ Health 11: 8, 2012.
9. Tsabouri S, Mavroudi A, Feketea G and Guibas GV: Subcutaneous and sublingual immunotherapy in allergic asthma in children. Front Pediatr 5: 8, 2017.
10. Strachan DP: Hay fever, hygiene, and household size. BMJ 299: 1259-1260, 1989.
11. Kim SH, Kim and Lee YC: Effect of *Coeni fructus* on ovalbumin-induced airway inflammation and airway hyper-responsiveness in a mouse of allergic asthma. J Inflamm (Lond) 9: 9, 2012.
12. Roviezzo F, Rossi A, Calazzo E, Orlando P, Riemma MA, Iacono VM, Guarino A, Ialenti A, Cicala C, Peritore A, *et al*: Palmitoylethanolamide supplementation during sensitization prevents airway allergic symptoms in the mouse. Front Pharmacol 8: 857, 2017.
13. Xu WH: Repetitive measurements of enhanced pause (Penh). Resp Physiol Neurobiol 206: 41-44, 2015.
14. Peng W, Qiu XQ, Shu ZH, Liu QC, Hu MB, Han T, Rahman K, Qin LP and Zheng CJ: Hepatoprotective activity of total iridoid glycosides isolated from *Paederia scandens* (Lour.) Merr. var. Tomentosa. J Ethnopharmacol 174: 317-321, 2015.
15. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
16. Liu B, Yuan J, Yin SM, Li Z, Xie Y, Chen Y, Shi Y, Zhang H, Li Y, Lam TW and Luo R: COPE: An accurate k-mer-based pair-end reads connection tool to facilitate genome. Bioinformatics 28: 2870-2874, 2012.
17. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, *et al*: Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75: 7537-7541, 2009.
18. Xu L: Animal models of human diseases. Dongwuxue Yanjiu 32: 1-3, 2011 (In Chinese).
19. Dai JH, Tan Y and Zhou F: The current situation of researches on animal model of asthma. Clin J Lab Animal Sci 11: 167-171, 2001.
20. Sjögren YM, Tomicic S, Lundberg A, Böttcher MF, Björkstén B, Sverremark-Ekström E and Jenmalm MC: Influence of early gut microbiota on the maturation of childhood mucosal and systemic immune responses. Clin Exp Allergy 39: 1842-1851, 2009.
21. Duffy L and O'Reilly SC: Toll-like receptors in the pathogenesis of autoimmune diseases: Recent and emerging translational developments. Immunotargets Ther 5: 69-80, 2016.
22. Achek A, Yesudhas D and Choi S: Toll-like receptors: Promising therapeutic targets for inflammatory diseases. Arch Pharm Res 39: 1032-1049, 2016.
23. Lee WS, Shin JS, Jang DS and Lee KT: Cnidilide, an alkylphthalide isolated from the roots of *Cnidium officinale*, suppresses LPS-induced NO, PGE2, IL-1β, IL-6 and TNF-α production by AP-1 and NF-κB inactivation in RAW 264.7 macrophages. Int Immunopharmacol 40: 146-155, 2016.
24. Im EJ, Kim SJ, Hong SB, Park JK and Rhee MH: Anti-inflammatory activity of Bee Venom in BV2 microglial cells: Mediation of MyD88-dependent NF-κB signaling pathway. Evid Based Complement Alternat Med 2016: 3704764, 2016.
25. Rana M, Maurya P, Reddy SS, Singh V, Ahmad H, Dwivedi AK, Dikshit M and Barthwal MK: A standardized chemically modified curcuma longa extract modulates IRAK-MAPK signaling in inflammation and potentiates cytotoxicity. Front Pharmacol 7: 233, 2016.
26. He A, Ji R, Shao J, He C, Jin M and Xu Y: TLR4-MyD88-TRAF6-TAK1 complex-mediated NF-κB activation contribute to the anti-inflammatory effect of V8 in LPS-induced human cervical cancer SiHa cells. Inflammation 39: 172-181, 2016.
27. Flaherty S and Reynolds JM: TLR function in murine CD4 (+) T lymphocytes and their role in inflammation. Methods Mol Biol 1390: 215-227, 2016.
28. Sabharwal H, Cichon C, Ölschläger TA, Sonnenborn U and Schmidt MA: Interleukin-8, CXCL1, and MicroRNA miR-146a responses to probiotic *Escherichia coli* nissle 1917 and enteropathogenic *E. coli* in human intestinal epithelial T84 and Monocytic THP-1 cells after apical or basolateral infection. Infect Immun 84: 2482-2492, 2016
29. Ma YH, Krikun G, Abrahams VM, Mor G and Guller S: Cell type-specific expression and function of toll-like receptor 2 and 4 in human placenta: Implications in fetal infection. Placenta 28: 1024-1031, 2007.
30. Ding Y, Liao W, He X, Xiang W and Lu Q: CSTMP exerts anti-Inflammatory effects on LPS-induced human renal proximal tubular epithelial cells by inhibiting TLR4-mediated NF-κB pathways. Inflammation 39: 849-859, 2016.
31. Conti F, Boucherit N, Baldassarre V, Trouplin V, Toman R, Mottola G, Mege JL and Ghigo E: Coxiella burnetii lipopolysaccharide blocks p38α-MAPK activation through the disruption of TLR-2 and TLR-4 association. Front Cell Infect Microbiol 4: 182, 2015.
32. DeWire SM, Ahn S, Lefkowitz RJ and Shenoy SK: Beta-arrestins and cell signaling. Ann Rev Physiol 69: 483-510, 2007.
33. Ma L and Pei G: Beta-arrestin signaling and regulation of transcription. J Cell Sci 120: 213-218, 2007.
34. Walker JK, Fong AM, Lawson BL, Savov JD, Patel DD, Schwartz DA and Lefkowitz RJ: Beta-arrestin-2 regulates the development of allergic asthma. J Clin Invest 112: 566-574, 2003.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.