Mechanism of Toll-like Receptor 4 in Host Immunity and Effects of Some Nutritional Factors: Postprint

Authors: Qin Long, Jiang Ning, Zhang Aizhong, Ma Di

Date: 2017-10-11T00:00:00+00:00

Abstract

Toll-like receptor 4 (TLR4) plays a crucial role in defending the organism against exogenous and endogenous antigenic challenges and serves as a bridge connecting innate and adaptive immunity. TLR4-mediated signaling pathways have become a hotspot in life science research in recent years. Studying TLR4 signaling pathways can provide in-depth elucidation of the organism' s immune mechanisms. This article analyzes recent domestic and international research on TLR4, comprehensively reviews its structure, distribution, ligands, and mechanisms of action, and prospects future development directions, aiming to provide theoretical references for future scientific research and medical practice.

Full Text

Mechanism of Toll-Like Receptor 4 in Immune Function and the Effects of Some Nutritional Factors on It

QIN Long, JIANG Ning*, ZHANG Aizhong, MA Di
College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing 163319, China

Abstract

Toll-like receptor 4 (TLR4) plays a crucial role in defending against exogenous and endogenous antigenic challenges and serves as a critical bridge connecting innate and adaptive immunity. TLR4-mediated signaling pathways have become a focal point of life sciences research in recent years, offering deep insights into immune mechanisms. This review analyzes recent domestic and international research on TLR4, summarizing its structure, distribution, ligands, and mechanisms of action, while also providing perspectives on future research directions to offer theoretical references for scientific research and medical applications.

Keywords: TLR4; signaling pathway; mechanism; immunity

Toll-like receptor 4 (TLR4) is the earliest discovered, most extensively studied, and most widely applied immune receptor within the Toll-like receptor (TLR) family. It functions as a bridge between innate and adaptive immunity, making substantial contributions to the establishment and maturation of the initial immune system. TLR4' s unique transmembrane structure enables cells to receive external signals and mount appropriate responses. Through interactions with intracellular signaling molecules, TLR4 amplifies external signals via cascade reactions, activating the nuclear factor- B (NF- B) pathway and stimulating nucleic acid expression to secrete cytokines for immune protection against exogenous pathogens. However, immune responses can also trigger detrimental inflammatory reactions resulting from excessive activation of signaling molecules in the TLR4/NF- B pathway. Targeting pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [1] to manipulate uncontrolled activation in this pathway and attenuate inflammatory processes represents a compelling approach for immunotherapy. To date, research on TLR4 mechanisms has advanced considerably, focusing primarily on immune surveillance, pathway guidance, and detection methods, though studies on its genetic aspects and applications remain scarce. This review synthesizes recent research on TLR4 and its mechanisms to provide new insights and theoretical references for future scientific directions.

1. Structure, Distribution, and Ligands of TLR4

The TLR4 gene is located at position 9 932 233, with a cDNA length of 3,811 bp, encoding a type I transmembrane protein composed of 879 amino acids that belongs to the pattern recognition receptor family. Its structure comprises three domains: extracellular, transmembrane, and intracellular. The extracellular domain consists of leucine-rich repeat (LRR) sequences forming a horseshoe-shaped structure containing two conserved modules and a ligand-binding region (LBR) that directly recognizes PAMPs. This region exhibits considerable variability during evolution, enabling recognition of diverse ligand molecules [2]. The transmembrane domain comprises a 21-amino-acid helix (primarily cysteine) that anchors TLR4 to the cell membrane and facilitates its proper localization.

The intracellular domain contains approximately 200 amino acid residues forming the highly conserved Toll/interleukin-1 receptor (TIR) domain, which represents the critical node for TLR4/NF- B signal activation and transduction. The TIR domain specifically recruits adaptor molecules myeloid differentiation primary response protein 88 (MyD88) and TIR-domain-containing adaptor inducing interferon-β (TRIF). Dimerization with adaptor molecule TIR domains initiates downstream signal transmission following TLR4 activation.

TLR4 is widely distributed throughout the animal body, serving as the primary

functional molecule in macrophages [3] and monocytes, with expression also detected in vascular smooth muscle cells [4], neutrophils [5], dendritic cells [6], small intestinal epithelial cells, gingival fibroblasts [7], cervical smooth muscle cells, respiratory epithelial cells, glial cells [8], spleen, and cardiac muscle cells. Research has shown that TLR4 gene silencing attenuates peroxiredoxin I (Prx I)-induced proliferation, differentiation, and migration of vascular smooth muscle cells [4], thereby affecting antioxidant stress capacity.

Lipopolysaccharide (LPS) represents the primary ligand for TLR4 and the main target of its immune surveillance, binding directly and specifically to TLR4 to activate the TLR4/NF- B signaling pathway. Additional pathogen-associated molecular patterns include lipid A, heat shock protein 60 (HSP60) [9], and paclitaxel (Taxol) [10]. Damage-associated molecular patterns constitute major TLR4 ligands that are released into intercellular spaces or circulation following tissue injury, hypoxia, or stress, thereby inducing autoimmunity or immune tolerance and playing important roles in arthritis, atherosclerosis, tumors, and systemic lupus erythematosus. During staphylococcal sepsis, TLR4 genetic variation correlates with cytokine levels produced in response to *Staphylococcus aureus*, and although this Gram-positive bacterium does not directly express LPS or activate TLR4, innate immune resistance to *S. aureus* appears TLR4-regulated, showing significant commonalities with Gram-negative bacteria and LPS [11].

2.1. Extracellular Signal Reception by TLR4

LPS, also known as bacterial endotoxin, is a macromolecule composed of lipid A and polysaccharides that forms the outer membrane of Gram-negative bacteria together with proteins and phospholipids. Lipid A serves as the toxic and biologically active center of LPS, possessing a stable structure without species specificity, thus producing similar toxic effects across different Gram-negative bacteria. Upon invasion, LPS on the bacterial outer membrane forms a protective barrier against antibiotics. LPS specifically binds to lipopolysaccharide-binding protein (LBP), which transports it to the immune cell membrane surface for binding with membrane protein CD14. LPS is then transferred to the LRR and myeloid differentiation-2 (MD-2) to form a protein complex. Using fluorescence resonance energy transfer technology in live cells, Zhong et al. [12] identified the Glu24–Met41 region of TLR4 as the MD-2 binding site. Following LPS binding, TLR4 undergoes activation, conformational changes, and dimerization —a process that typically lasts only about four minutes.

2.2. Intracellular Signal Transduction by TLR4

Intracellular signal transduction occurs through both MyD88-dependent and -independent pathways. MyD88 consists of a short amino acid sequence connecting an N-terminal death domain (DD) and a C-terminal TIR domain [13]. Upon TLR4 binding to PAMPs, receptor dimerization occurs, and the MyD88

C-terminus interacts with TLR structures in the cytoplasm. The N-terminal DD recruits downstream serine/threonine protein kinases with death domains, interleukin-1 receptor-associated kinase 1 (IRAK1) and IRAK2, enabling their autophosphorylation. Phosphorylated IRAK dissociates from MyD88 to bind and activate tumor necrosis factor receptor-associated factor 6 (TRAF6), initiating two distinct signaling cascades: one involving p38 [mitogen-activated protein kinase (MAPK) family], c-Jun N-terminal kinase (JNK), and interferon regulatory factor (IRF) transcription factor family (IRF-5) that directly induces pro-inflammatory cytokine expression; the other activating the inhibitor of nuclear factor- B kinase (IKK) complex, which phosphorylates and activates IKK to bind NF- B, inducing nucleic acid expression that leads to production of specific pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and matrix metalloproteinase (MMP), thereby completing signal transduction (Figure 1 [Figure 1: see original paper]). The MyD88-dependent pathway plays important roles in many inflammatory responses, including cigarette smoke-induced pulmonary inflammation [14].

TLR4' s intracellular TIR domain also connects with the TRIF-related adaptor molecule (TRAM), which activates TRIF to bind TRAF6 and transmit signals to IRF-3. Following phosphorylation, IRF-3 forms homodimers that translocate into the nucleus to induce interferon (IFN) gene expression. TLR4 signaling activity is regulated by cholesterol levels in the cell membrane, which are influenced by bis(monoacylglycero)phosphate (BMP). Ciesielska et al. [15] found that incorporating exogenous BMP isoforms into macrophage plasma membranes and intracellular vesicles significantly reduced LPS-stimulated chemokine production through inhibition of IRF-3, which controls chemokine expression. Although simpler than the MyD88-dependent pathway, the MyD88-independent pathway plays crucial roles by inducing IFN production. As a broad-spectrum antiviral agent, IFN does not directly kill or inhibit viruses but rather binds to cell surface receptors to induce antiviral proteins that enhance cellular immunity.

TRAF6: tumor necrosis factor receptor-associated factor 6; **MAPK:** mitogen-activated protein kinase; **JNK:** c-Jun N-terminal kinase; **IRF:** interferon regulatory factor; **IFN:** interferon; **IKK:** inhibitor of nuclear factor- B kinase; **I B:** inhibitor of nuclear factor- B; **NF- B:** nuclear factor- B; **TNF:** tumor necrosis factor; **IL-1:** interleukin-1; **MMP:** matrix metalloproteinase; **TIR:** Toll/interleukin-1 receptor; **TRAM:** TRIF-related adaptor molecule; **MyD88:** myeloid differentiation primary response protein 88; **TRIF:** TIR-domain-containing adaptor inducing interferon-β; **IRF-3:** interferon regulatory factor-3; **P:** phosphorylation; **LPS:** lipopolysaccharides; **LBP:** lipopolysaccharide binding protein; **LRR:** leucine-rich repeat; **MD-2:** myeloid differentiation-2.

Figure 1. TLR4-mediated signaling pathway.

2.3. Inhibition of Signaling Pathways by TLR4 Blockers

TLR4 blockers are classified into two types based on their action sites. The first type blocks the specific binding of LPS to TLR4, preventing TLR4 activation and keeping signaling proteins in an inhibited state. Oxidized phospholipids can block LPS binding to LBP and CD14, protecting against LPS-induced tissue damage [16]. The purified TLR4 monoclonal antibody MTS510 from mouse ascites has been shown to recognize and block the TLR4-CD14 complex, inhibiting NF- B translocation and pro-inflammatory cytokine production [17]. The second type blocks pro-inflammatory signal transmission by inhibiting signaling proteins in the pathway, including MyD88 inhibitors [18], IRAK inhibitors [19], IKK blockers [20], and NF- B inhibitors [21], thereby suppressing and negatively regulating the pathway to prevent autoimmune diseases caused by excessive TLR4 activation. Given the important role of TLR4-mediated signaling in various inflammatory pathogenesis mechanisms, targeted inhibition or blocking at specific sites holds promise for future treatment of inflammatory diseases.

2.4. TLR4 as a Bridge Between Innate and Adaptive Immunity

In innate immune responses, TLR4 participates in recognizing Gram-negative bacteria during initial invasion, transmitting signals downstream via the TLR4 signaling pathway to activate NF- B, which translocates into the nucleus to initiate transcription and translation of bactericidal substances and pro-inflammatory cytokines. As one of the primary sensors in innate immunity, TLR4 plays a crucial role under pathological conditions such as inflammatory bowel disease, largely determining the initiation, intensity, scope, and progression of innate immune responses, thus holding an irreplaceable position [22]. Effector molecules of innate immunity primarily consist of antimicrobial peptides, among which defensins are most extensively studied. Defensins are broad-spectrum antimicrobial peptides that kill bacteria by disrupting their cell membranes [23]. Innate immunity requires a series of physiological and biochemical reactions, often taking considerable time and causing tissue damage through inflammatory responses. Subsequently, the body requires a rapid and precise immune mechanism to defend against re-invasion by the same antigens. During innate immune responses, TLR4 activation by LPS releases cytokines through signaling pathways that stimulate adaptive immunity maturation. Immature CD4+ helper T cells differentiate into two functionally distinct subsets, Th1 and Th2. Th1 cytokine IFN-γ promotes the TLR4/MD-2 signaling pathway in intestinal epithelial cells, enhancing LPS-induced secretion of the pro-inflammatory cytokine interleukin-8 (IL-8) [24]. The regulation of adaptive immunity by innate immunity through TLR4 underscores its critical role in the immune system. Macrophages constitute the first line of defense against *Mycobacterium tuberculosis* and play an important role in bridging innate and adaptive immunity. The novel macrophage-activating protein Rv2882c activates the TLR4/NF- B pathway to secrete pro-inflammatory cytokines, and

Rv2882c-treated macrophages induce expansion of effector or memory T cell populations and Th1 immune responses [25]. Studies have shown that Prx provides protective effects during LPS-induced mouse immune responses, with significant changes in cytokine levels produced by the TLR4/NF- B signaling pathway, demonstrating TLR4' s positive roles in antioxidant stress, apoptosis, proliferation, differentiation, and migration [26].

2.5. Targeted TLR4-Based Disease Therapy

Inhibition and blocking within the TLR4 signaling transduction pathway represent a therapeutic approach achieved primarily through suppressing and competing with TLR4 signaling. Many related products have already entered practical application. Shen et al. [27] reported that the TLR4 small-molecule inhibitor TAK-242 protects against renal ischemia-reperfusion injury by attenuating TLR4/NF- B-mediated inflammatory responses. The TLR4 signaling pathway also participates in probiotic modulation of intestinal flora. Liu et al. [28] found that TLR4 signaling is involved in both the pathogenic mechanism of *Helicobacter pylori* (Hp) infection and probiotic treatment of Hp. TLR4 expression levels showed no correlation with gastric mucosal inflammation pathology scores, suggesting TLR4 may not participate in maintaining inflammatory responses but rather completes its signal transmission function before downstream inflammatory factors [such as interleukin-1β (IL-1β)] take over the cascade amplification. Macrophages infected with human immunodeficiency virus (HIV) show reduced viral replication when exposed to Gram-negative bacteria, with viral replication restored upon bacterial removal, demonstrating TLR4' s important role in bacterial inhibition by phagocytes [29]. Additionally, TLR4 ligands are widely applied as vaccine adjuvants, where their combination with vaccine antigens enhances and modifies immune responses, constituting a new generation of adjuvants [30].

3.1. Role in Antimicrobial Peptide Bacteriostatic Mechanisms

Modern molecular biology and genetic engineering technologies have enabled the rational design of antimicrobial peptides, which have found extensive applications across numerous fields. Antimicrobial peptides typically exhibit secondary structures that target bacterial cell membranes, causing irreversible depolarization and membrane disruption to exert full antibacterial activity. This membrane destruction releases bacterial endotoxins that stimulate macrophages to produce endogenous pyrogens including INF, IL-1, and interleukin-2 (IL-2), triggering strong febrile responses. Macrophage membrane TLR4 recognizes these endotoxins and conducts signal transduction, leading to release of various cytokines that upregulate costimulatory molecules on antigen-presenting cells and induce specific immunity for endotoxin phagocytosis and degradation. However, excessive TLR4 activation causes upregulated cytokine expression beyond immune system control, resulting in autoimmune diseases—a phenomenon

frequently observed in young animals with immature immune systems where excessive cytokine release from various stressors often leads to systemic immune disorders.

3.2. Role in Intestinal Immune Barrier Formation

The intestinal immune barrier comprises gut-associated lymphoid tissue, diffuse immune cells, and immune-active substances such as secretory immunoglobulin A (sIgA), representing the most important and complex component of the immune system. This complexity arises because the intestine serves as the body' s primary interface with the external environment, where nutrient absorption, biochemical reactions, bacterial symbiosis, and antigen invasion converge. Moreover, the intestine' s synergistic interactions with other immune tissues and its own immune functions, along with mucosal-neuro-immune and endocrine-immune networks [31], collectively protect organismal health.

Intestinal epithelial lymphocytes, dendritic cells, and M cells in the intestinal mucosa all express TLR4, which distinguishes harmful antigens from harmless substances to continuously monitor the intestinal environment. Upon recognizing and binding harmful antigens (primarily LPS), TLR4 signaling induces effector cells to produce immune-active substances for antigen processing. As adaptive immunity gradually matures and becomes predominant, re-exposure to antigens triggers rapid, accurate, specific antibody-mediated elimination. This process ensures successful weaning in young animals, and accelerating adaptive immunity maturation facilitates early weaning, reducing rearing costs and improving production performance.

3.3. Role in the Brain-Gut Axis Model

The brain-gut axis represents a connection between the central and enteric nervous systems, prominently manifested in chemotherapy treatments where neural drug administration causes excessive expression of endogenous danger signals, significantly inducing intestinal toxicity [32]. Recent studies indicate that TLR4 expression in intestinal epithelial cells during chemotherapy is closely related to intestinal toxicity through activation of downstream NF- B and induction of immune responses [33]. Concurrently, intestinal inflammation also regulates the central nervous system through TLR4 (Figure 2 [Figure 2: see original paper]).

GIT: gastrointestinal tract; **CNS:** central nervous system; **IL-1:** interleukin-1; **TNF:** tumor necrosis factor; **MMP-2:** matrix metalloproteinase-2; **IL-6:** interleukin-6; **MMP-9:** matrix metalloproteinase-9; **Blood Brain Barrier:** blood-brain barrier; **Caveolae:** caveolae; **Tight junction:** tight junction; **Glial cell (microglia/astrocyte):** glial cell (microglia/astrocyte); **Neuronal terminal:** neuronal terminal; **TLR4:** Toll-like receptor 4; **ROS:** reactive oxygen species; **Chemokines:** chemokines; **Pain:** pain.

Figure 2. Intestine regulates the central nervous system through TLR4 [34].

The brain-gut axis model has become a popular research topic in recent years, with its mechanisms still under investigation. Intestinal flora and immunity will undoubtedly be key focuses in elucidating these mechanisms.

3.4. Role in Nutritional Factor Regulation Mechanisms

TLR4 participates in the regulatory mechanisms of many nutritional factors, enabling its expression level to serve as a measure of dietary supplementation levels. Research reports that antimicrobial peptides reduce expression of TLR4 signaling upstream regulatory proteins TLR4 and MyD88, as well as phosphorylation levels of inflammation-related proteins NF- B and inhibitor-α of nuclear factor- B (I B-α) in jejunal tissue, thereby alleviating intestinal inflammation [35]. However, excessive antimicrobial peptide supplementation inevitably decreases TLR4 sensitivity to PAMPs and may even abolish recognition function, causing toxic reactions. Vitamins enhance innate immunity, with vitamin D augmenting antimicrobial capacity in monocytes and macrophages through TLR4 modulation. The active form of vitamin D, 1,25-dihydroxyvitamin D3, downregulates TLR4 expression in cultured human monocytes, rendering them hyporesponsive to PAMPs and preventing excessive TLR4 activation and inflammation [36]. TLR4 also participates in fatty acid regulation of agouti gene-related protein (AgRP) expression and secretion in N38 cells, affecting feeding behavior and energy balance, though the mechanisms remain unclear. Studies indicate that TLR4 not only induces insulin resistance and metabolic disorders through inflammatory responses under pathological conditions but also plays important regulatory roles in glucose and lipid metabolism during physiological starvation states [37], directly interacting with nutritional factors to maintain metabolic homeostasis.

This review has analyzed TLR4 signal transduction and regulation, introduced its bridging role between innate and adaptive immunity, listed disease therapies targeting TLR4 signaling, and described its functions in antimicrobial peptide mechanisms, intestinal mucosal immunity, and brain-gut axis models. TLR4 signaling is crucial for perfecting adaptive immunity before weaning in young animals, and future in-depth studies on this pathway could effectively protect the health of early-weaned animals and improve production performance. We propose the following future directions: (1) Manipulating TLR4 activation to regulate inflammatory processes and perfect immune system development will become a major research trend, given its bridging role in innate and adaptive immunity; (2) The TLR4/NF- B pathway is widely recognized as a classical pro-inflammatory pathway with abundant research on inhibitors and blockers, making the duration of inhibitory/blocking effects a key focus; (3) Antimicrobial peptides are considered ideal antibiotic alternatives due to their broad-spectrum antibacterial activity and lack of residues, with current research focusing on polycationic peptides such as magainin, cecropin, and housefly antimicrobial peptides that bind targets primarily through electrostatic adsorption, which is insufficient for targeted therapy. Recent studies on cloning, expression, and

functional activity of TLR4 extracellular domains suggest that tandem modification of TLR4 extracellular domain genes with antimicrobial peptide genes could enhance targeted recognition of Gram-negative bacteria, representing a promising research direction.

References

[1] GUSTAVSEN A, NYMO S, LANDSEM A, et al. Combined inhibition of complement and CD14 attenuates bacteria-induced inflammation in human whole blood more efficiently than antagonizing the toll-like receptor 4-MD2 complex[J]. *The Journal of Infectious Diseases*, 2016, 214(1): 140–150.

[2] AKIRA S, UEMATSU S, TAKEUCHI O. Pathogen recognition innate immunity[J]. *Cell*, 2006, 124(4): 783–801.

[3] CHATTERJEE B, BANOTH B, MUKHERJEE T, et al. Late-phase synthesis of I Bα insulates TLR4-activated canonical NF- B pathway from noncanonical NF- B signaling in macrophages[J]. *Science Signaling*, 2016, 9(457): ra120.

[4] ZHU Z C, ZHENG X M, LI D, et al. Prx1 promotes the proliferation and migration of vascular smooth muscle cells in a TLR4-dependent manner[J]. *Molecular Medicine Reports*, 2017, 15(1): 345–351.

[5] BARRANCO C. Inflammation: soluble MRP8/14 recruits neutrophils via TLR4[J]. *Nature Reviews Rheumatology*, 2015, 11(6): 320.

[6] SAHASRABUDHE N M, DOKTER-FOKKENS J, DE VOS P. Particulate β-glucans synergistically activate Dectin-1 human dendritic cells[J]. *Molecular Nutrition & Research*, 2016, 60(11): 2514–2522.

[7] LAPPIN M J, BROWN V, ZARIC S S, et al. Interferon-γ stimulates CD14, TLR2 and TLR4 mRNA expression in gingival fibroblasts increasing responsiveness to bacterial challenge[J]. *Archives of Oral Biology*, 2016, 61: 36–43.

[8] LI X Q, ZHANG Z L, TAN W F, et al. Down-regulation of CXCL12/CXCR4 expression alleviates ischemia-reperfusion-induced inflammatory pain via inhibiting glial TLR4 activation in the spinal cord[J]. *PLoS One*, 2016, 11(10): e0163807.

[9] SWAROOP S, SENGUPTA N, SURYAWANSHI A R, et al. HSP60 plays a regulatory role in IL-1β-induced microglial inflammation via TLR4-p38 MAPK axis[J]. *Journal of Neuroinflammation*, 2016, 13: 27.

[10] GE X, CAO Z, GU Y, et al. PFKFB3 potentially contributes to paclitaxel resistance in breast cancer cells through activation stimulating lactate production[J]. *Molecular & Cellular Biology*, 2016, 62(6): 119–125.

[11] CHANTRATITA N, TANDHAVANANT S, SEAL S, et al. TLR4 genetic variation is associated with inflammatory responses Gram-positive sepsis[J]. *Clinical Microbiology Infection*, 2017, 23(1): 47.e1–47.e10.

[12] ZHONG T Y, TANG J, CHEN D Y, et al. Study on the interaction domain between TLR4 and MD-2 in living cells using fluorescence resonance energy transfer technology[J]. *Progress in Biochemistry and Biophysics*, 2009, 36(11): 1451–1457.

[13] MCGETTRICK A F, O' NEILL L A. The expanding family of MyD88-like adaptors in Toll-like receptor signal transduction[J]. *Molecular Immunology*, 2004, 41(6/7): 577–582.

[14] CHENG Y, WANG D, WANG B, et al. HMGB1 translocation and release mediate cigarette smoke-induced pulmonary inflammation in mice through a TLR4/MyD88-dependent signaling pathway[J]. *Molecular Biology of the Cell*, 2017, 28(1): 201–209.

[15] CIESIELSKA A, SAS-NOWOSIELSKA H, KWIATKOWSKA K. Bis (monoacylglycero) phosphate inhibits TLR4-dependent RANTES production in macrophages[J]. *The International Journal of Biochemistry & Cell Biology*, 2016, 83: 15–26.

[16] BOCHKOV V N, KADL A, HUBER J, et al. Protective role of phospholipid oxidation products in endotoxin-induced tissue damage[J]. *Nature*, 2002, 419(6902): 77–81.

[17] AKASHI S, SHIMAZU R, OGATA H, et al. Cutting edge: cell surface expression and lipopolysaccharide signaling via the Toll-like receptor 4-MD-2 complex on mouse peritoneal macrophages[J]. *Journal of Immunology*, 2000, 164(7): 3471–3475.

[18] THOMSON A W. MyD88 inhibitors continuing challenge antagonism[J]. *Transplantation*, 2017, 101(2): 230–231.

[19] WANG Z L, WESCHE H, STEVENS T, et al. IRAK-4 inhibitors for inflammation[J]. *Current Topics in Medicinal Chemistry*, 2009, 9(8): 724–737.

[20] MOARBESS G, GUICHOU J F, PANIAGUA-GAYRAUD S, et al. New IKK inhibitors: synthesis of new imidazo[1,2-a]quinoxaline derivatives using microwave assistance and biological evaluation as IKK inhibitors[J]. *European Journal of Medicinal Chemistry*, 2016, 115: 268–274.

[21] JO H, CHOI M, KUMAR A S, et al. Development of novel 1,2,3,4-tetrahydroquinoline scaffolds as potent NF- B inhibitors and cytotoxic agents[J]. *ACS Medicinal Chemistry Letters*, 2016, 7(4): 385–390.

[22] LI G X, LI N. Research progress on Toll-like receptors in intestinal mucosal barrier immune mechanisms[J]. *Parenteral & Enteral Nutrition*, 2008, 15(6): 366–368, 372.

[23] HU Q F, CHEN T X. Research progress on defensins[J]. *Journal of Clinical Pediatrics*, 2015, 33(9): 831–834.

[24] MUELLER T, TERADA T, ROSENBERG I M, et al. Th2 cytokines down-regulate TLR expression and function in human intestinal epithelial cells[J].

Journal of Immunology, 2006, 176(10): 5805-5814.

[25] CJOI H G, CHOI S, BACK Y W, et al. Mycobacterium tuberculosis Rv2882c protein induces activation of macrophages through TLR4 and exhibits vaccine potential[J]. *PLoS One*, 2016, 11(10): e0164458.

[26] FENG L. Study on the regulatory role of Prx in LPS-induced immune responses in mice[D]. Master' s thesis. Daqing: Heilongjiang Bayi Agricultural University, 2015.

[27] SHEN Y Y, GUO S X. Protective effect of Toll-like receptor 4 inhibitor on renal ischemia-reperfusion injury in rats[J]. *Zhejiang Journal of Traumatic Surgery*, 2015(6): 1082-1085.

[28] LIU X, LIN M P, DING Y W, et al. Role of TLR4 in probiotic treatment of Hp infection in mice[J]. *China Journal of Modern Medicine*, 2011, 21(1): 32-35, 40.

[29] AGOSTO L M, HIRENT J B, MICHAELS D H, et al. Porphyromonas gingivalis-mediated signaling through TLR4 mediates persistent HIV infection of primary macrophages[J]. *Virology*, 2016, 499: 72-81.

[30] CARTER D, FOX C B, DAY T A, et al. A structure-function approach to optimizing TLR4 ligands for human vaccines[J]. *Clinical & Translational Immunology*, 2016, 5(11): e108.

[31] ZHOU Z H, MA L L. Relationship between irritable bowel syndrome and neuro-endocrine-immune network[J]. *Chinese Journal of Integrated Traditional and Western Medicine on Digestion*, 2013, 21(2): 106-109.

[32] BIANCHI M E. DAMPs, PAMPs and alarmins: all we need to know about danger[J]. *Journal of Leukocyte Biology*, 2007, 81(1): 1-5.

[33] HAMADA K, KAKIGAWA N, SEKINE S, et al. Disruption of ZO-1/claudin-4 interaction in relation to inflammatory responses in methotrexate-induced intestinal mucositis[J]. *Cancer Chemotherapy and Pharmacology*, 2013, 72(4): 757-765.

[34] WARDILL H R, VAN SEBILLE Y Z, MANDER K A, et al. Toll-like receptor 4 signaling: a common biological mechanism of regimen-related toxicities: an emerging hypothesis for neuropathy and gastrointestinal toxicity[J]. *Cancer Treatment Reviews*, 2015, 41(2): 122-128.

[35] YI H B. Effects and mechanisms of antimicrobial peptide CWA on intestinal inflammation and barrier function in weaned piglets[D]. Doctoral dissertation. Zhejiang: Zhejiang University, 2016.

[36] SADEGHI K, WESSNER B, LAGGNER U, et al. Vitamin D3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns[J]. *European Journal of Immunology*, 2006, 36(2): 361-370.

[37] PANG S S, LE Y Y. Regulatory role of Toll-like receptor 4 in starvation metabolism[J]. *China Basic Science*, 2011, 13(3): 30-32.

Note: Figure translations are in progress. See original paper for figures.