Mechanism of Toll-like Receptor 4 in Host Immunity and the Influence of Nutritional Factors: A Postprint

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Abstract

Toll-like receptor 4 (TLR4) makes important contributions to defending the organism against exogenous and endogenous antigenic challenges, serves as a bridge connecting innate and adaptive immunity, and TLR4-mediated signaling pathways have been a hot topic in life science research in recent years. By studying TLR4 signaling pathways, we can elucidate immune mechanisms in depth. This article analyzes recent domestic and international research on TLR4, reviews its structure, distribution, ligands, and mechanisms of action, and discusses 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

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Abstract

Toll-like receptor 4 (TLR4) plays a crucial role in defending against exogenous and endogenous antigenic challenges and serves as a bridge connecting innate and adaptive immunity. The TLR4-mediated signaling pathway has become a focal point of life sciences research in recent years, offering deep insights into immune mechanisms. This paper reviews recent domestic and international research on TLR4, summarizing its structure, distribution, ligands, and mechanisms of action, and discusses future research directions to provide a theoretical foundation for scientific research and medical applications.

Keywords: TLR4; signaling pathway; mechanism; immunity

Toll-like receptor 4 (TLR4) is the most extensively studied and widely applied immune receptor in the Toll-like receptor family, serving as a critical bridge between innate and adaptive immunity and contributing significantly to the establishment and refinement 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 activation of the nuclear transcription factor- B (NF- B) pathway, stimulating nucleic acid expression and cytokine secretion for immune protection against exogenous pathogens. However, excessive activation of signaling molecules in the TLR4/NF- B pathway can trigger detrimental inflammatory responses. Targeting upstream pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [1] to control uncontrolled activation and attenuate inflammatory processes represents a promising approach for immunotherapy. To date, research on TLR4 mechanisms has advanced considerably, focusing primarily on immune surveillance, pathway regulation, and detection methods, though studies on its genetic aspects remain limited. This review summarizes recent research on TLR4 and its mechanisms to provide new insights and theoretical references for future investigations.

1 Structure, Distribution, and Ligands of TLR4

The TLR4 gene is located at position 9q32.2-33, 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 region consists of leucine-rich repeat (LRR) sequences forming a horseshoe-shaped domain with 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 region comprises a 21-amino-acid helix (primarily cysteine) that anchors TLR4 to the cell membrane and facilitates its localization. The intracellular region contains a highly conserved Toll/interleukin-1 receptor (TIR) domain of approximately 200 amino acid residues, which is critical for TLR4/NF- B pathway activation and signal transduction. The TIR domain specifically recruits adaptor molecules myeloid differentiation primary response protein 88 (MyD88) and TIR-domain-containing adaptor inducing interferon- (TRIF), with dimerization of the TIR domains initiating downstream signal transmission upon TLR4 activation.

TLR4 is widely distributed throughout the animal body, serving as a key molecule in macrophages [3] and monocytes, and is also expressed in vascular smooth muscle cells [4], neutrophils [5], dendritic cells [6], intestinal epithelial

cells, gingival fibroblasts [7], cervical smooth muscle cells, respiratory epithelial cells, glial cells [8], spleen, and cardiac muscle cells. Studies have shown that TLR4 gene silencing attenuates peroxiredoxin I (Prx I)-induced proliferation, differentiation, and migration of vascular smooth muscle cells [4], affecting antioxidant stress capacity.

Lipopolysaccharides (LPS) are the primary ligand of TLR4 and the main target of its immune surveillance, directly binding to TLR4 to activate the TLR4/NF-B signaling pathway. Additional ligands include lipid A, heat shock protein 60 (HSP60) [9], paclitaxel (Taxol) [10], and various other pathogen-associated molecular patterns. Damage-associated molecular patterns represent major TLR4 ligands that are released into intercellular spaces or circulation following tissue damage, 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 *S. aureus* does not directly express LPS or activate TLR4, innate immune resistance to this pathogen appears TLR4-regulated and shares 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 represents the toxic and biologically active center of LPS with a stable, non-species-specific structure, resulting in similar toxic effects across different Gram-negative bacteria. Upon bacterial invasion, LPS forms a protective barrier around bacteria to evade antibiotics. LPS specifically binds to lipopolysaccharide-binding protein (LBP) and is transported to the immune cell membrane surface, where it associates with membrane protein CD14 before being 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 approximately four minutes.

2.2 Intracellular Signal Transduction by TLR4

Intracellular signal transduction occurs through both MyD88-dependent and -independent pathways. MyD88 consists of a death domain (DD) at the N-terminus and a TIR domain at the C-terminus connected by a short amino acid sequence [13]. Upon PAMP binding, TLR4 dimerizes, and the MyD88 C-terminus interacts with the TLR structure 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, promoting their

autophosphorylation. Phosphorylated IRAK dissociates from MyD88, binding to and activating tumor necrosis factor receptor-associated factor 6 (TRAF6). Two distinct signal transduction pathways then emerge: 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), directly inducing 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 and leading to production of 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].

The TLR4 intracellular TIR domain also connects to Toll-like receptor-related molecule (TRAM), which acts on TRIF, promoting its activation and binding to TRAF6. The signal is then transmitted to IRF-3, which forms homodimers upon phosphorylation, translocates to the nucleus, and induces interferon (IFN) gene expression. TLR4 signaling activity is regulated by cholesterol levels in the cell membrane and consequently 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. Compared with the MyD88-dependent pathway, the MyD88-independent pathway is simpler yet critically important, 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 and enhance cellular immunity.

2.3 Inhibition of TLR4 Signaling Pathways by TLR4 Blockers

TLR4 blockers are classified into two types based on their target sites. The first type blocks specific LPS-TLR4 binding, preventing TLR4 activation and maintaining 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 induction [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 pathogeneses, targeted inhibition or blockade at specific sites will facilitate future treatment of inflammatory diseases.

2.4 TLR4 as a Link 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 pathway to activate NF- B, which translocates to 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 critical role under pathological conditions such as inflammatory bowel disease, largely determining the initiation, intensity, scope, and progression of innate immune responses [22]. Effector molecules of innate immunity primarily consist of antimicrobial peptides, with defensins being the most 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 accurate immune mechanism to defend against re-invasion by the same antigen. During innate immune responses, TLR4 activation by LPS releases cytokines via signaling pathways that stimulate adaptive immunity maturation. Immature CD4+ helper T cells differentiate into two functionally distinct subsets, Th1 and Th2. Th1 cytokine IFN- enhances the TLR4/MD-2 signaling pathway in intestinal epithelial cells, increasing 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 crucial role in the immune system. Macrophages constitute the first line of defense against *Mycobacterium tuberculosis* and play an important role in linking 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 I provides protective effects in LPS-induced mice while significantly altering cytokine levels produced through the TLR4/NF- B pathway, demonstrating TLR4' s positive roles in antioxidant stress, apoptosis, proliferation, differentiation, and migration [26].

2.5 Disease Therapy Targeting TLR4

Inhibiting and blocking the TLR4 signaling transduction pathway represents a therapeutic approach primarily achieved through competitive inhibition. Many related products have already been applied in practice. 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 is also involved in probiotic regulation of intestinal flora. Liu et al. [28] found that TLR4 participates in both the pathogenic mechanism of *Helicobacter pylori* (Hp) infection and the therapeutic mechanism of probiotics against Hp. TLR4 expression levels are unrelated to gastric mucosal inflammatory pathology scores, suggesting that TLR4 may not participate in maintaining inflammatory reactions but rather

completes its signal transmission function, with downstream inflammatory factors such as interleukin-1 (IL-1) responsible for cascade amplification. When phagocytes infected with human immunodeficiency virus (HIV) are exposed to Gram-negative bacteria, viral replication in cells decreases; however, removal of Gram-negative bacteria restores viral replication, indicating TLR4' s important role in bacterial inhibition by phagocytes [29]. Furthermore, TLR4 ligands are widely used as vaccine adjuvants, and their combination with vaccine antigens enhances and modifies immune responses, leading to a new generation of adjuvants [30].

3.1 Role in Antimicrobial Peptide Bacteriostatic Mechanisms

Modern molecular biology and genetic engineering technologies have enabled qualitative design of antimicrobial peptides, which have been widely applied across numerous fields. Antimicrobial peptides typically exhibit secondary structures that target bacterial cell membranes, causing irreversible depolarization and disruption to exert antibacterial activity. This membrane destruction releases bacterial endotoxins, which stimulate macrophages to produce endogenous pyrogens such as IFN, IL-1, and interleukin-2 (IL-2), triggering strong febrile responses. Macrophage membrane TLR4 recognizes these endotoxins and conducts signal transduction, causing 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 upregulates cytokine expression beyond immune system control, causing autoimmune diseases—a phenomenon particularly common in young animals with immature immune systems and underdeveloped specific immunity, 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 is the body's primary interface with the external environment, where nutrient absorption, biochemical reactions, bacterial symbiosis, and antigen invasion converge. The intestine also collaborates with other immune tissues, and intestinal mucosal immunity interacts with neuro-immunity and endocrine-immunity to form the body' s immune network [31], collectively protecting organismal health.

Intestinal epithelial lymphocytes, dendritic cells, and M cells in the intestinal mucosa all express TLR4, which distinguishes harmful antigens from harmless substances, continuously monitors the intestinal environment, and recognizes harmful antigens (primarily LPS). Signal transduction through the pathway in-

duces effector cells to produce immune-active substances for antigen processing. As adaptive immunity gradually develops and becomes dominant, re-exposure to antigens triggers rapid, accurate, specific antibody-mediated elimination, enabling early weaning in young animals. Accelerating adaptive immunity maturation in young animals 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 induces excessive expression of endogenous danger signals, significantly causing intestinal toxicity [32]. Recent studies indicate that TLR4 expression in intestinal epithelial cells during chemotherapy correlates closely with intestinal toxicity, activating downstream NF- B and generating immune responses [33]. Concurrently, intestinal inflammation also regulates the central nervous system through TLR4 (Figure 2 [Figure 2: see original paper]).

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 be indispensable focal points in mechanistic studies.

3.4 Role in Nutritional Factor Regulatory Mechanisms

TLR4 participates in the regulatory mechanisms of many nutritional factors, enabling its expression level to serve as a metric for nutritional factor supplementation in animal diets. Research reports that antimicrobial peptides reduce expression of TLR4 signaling pathway upstream regulatory proteins TLR4 and MyD88, as well as phosphorylation levels of inflammation-related proteins NF- B and inhibitor- of nuclear factor- B (I B-), thereby alleviating intestinal inflammation [35]. However, excessive antimicrobial peptide addition inevitably decreases TLR4 sensitivity to PAMPs or even abolishes its recognition function, causing toxic reactions. Vitamins enhance innate immunity; vitamin D enhances antimicrobial capacity of monocytes and macrophages by regulating TLR4 expression. 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 inflammatory responses [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 mechanism remains unclear. Research indicates 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 nutritional balance.

Conclusion and Future Directions

This review has analyzed TLR4 signal transduction and regulation, introduced its bridging role between innate and adaptive immunity, listed disease therapies targeting the TLR4 pathway, and described TLR4 functions in antimicrobial peptide mechanisms, intestinal mucosal immunity, and brain-gut axis models. The TLR4 signaling pathway is crucial for adaptive immunity maturation in young animals before weaning. Future in-depth research on this pathway could effectively protect the health of early-weaned young animals and improve production performance. We propose the following future directions: (1) TLR4' s bridging role between innate and adaptive immunity makes manipulating its activation to regulate inflammatory processes and perfect immune systems a future research trend; (2) The TLR4/NF- B pathway is widely recognized as a classic pro-inflammatory pathway with abundant inhibitor and blocker research, where sustained inhibitory effects will be a future 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 musca domestica antimicrobial peptides that bind targets primarily through electrostatic adsorption, showing limited targeting capability. Recent studies on cloning, expression, and functional activity of the TLR4 extracellular domain 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.

Abbreviations

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-
IRF-3: interferon regulatory factor-3
P: phosphorylation
LPS: lipopolysaccharides

LBP: lipopolysaccharide binding protein
LRR: leucine-rich repeat
MD-2: myeloid differentiation-2

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 B 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 -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- 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-1 -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 live 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 receptor mechanisms in intestinal mucosal barrier immunity[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 I in LPS-induced immune response 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 Trauma 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.

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