Toll-like Receptors and Their Regulatory Effects on Intestinal Mucosal Immunity (Postprint)

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Abstract

Toll-like receptors (TLRs) are pattern recognition receptors that have attracted considerable attention in recent years and function as pathogen sensors in both vertebrates and invertebrates. Recognition of specific intra- and extracellular ligands by TLRs is fundamental to initiating innate immunity, rapidly augmenting protective responses against invading pathogens, and ultimately activating adaptive immunity. TLRs play a crucial role in the discrimination between pathogenic and probiotic bacteria during intestinal immune responses; furthermore, TLRs can regulate the secretion of antimicrobial peptides by the intestinal epithelium to eliminate pathogenic bacteria, thereby exerting beneficial effects on intestinal health. This article introduces the classification of TLRs, their ligands, and the corresponding signaling pathways, and explores the pivotal role of TLRs in the regulation of intestinal immunity.

Full Text

Toll-Like Receptors and Their Regulatory Role in Intestinal Mucosal Immunity

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Abstract

Toll-like receptors (TLRs) are pattern recognition receptors that have attracted considerable attention in recent years, functioning as pathogen sensors in both vertebrate and invertebrate species. The recognition of specific ligands by TLRs forms the basis of innate immune activation, enabling rapid protective responses

against invading pathogens and ultimately triggering adaptive immunity. TLRs play a crucial role in distinguishing between pathogenic and probiotic bacteria during intestinal immune responses, while also regulating the secretion of antimicrobial peptides from intestinal epithelial cells to eliminate pathogens, thereby exerting positive effects on gut health. This review introduces the types of TLRs, their ligands, and corresponding signaling pathways, and discusses the pivotal role of TLRs in regulating intestinal immunity.

Keywords: Toll-like receptors; intestinal mucosal immunity; ligands; signal transduction; antimicrobial peptides

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Initially identified as membrane surface receptors controlling dorsoventral differentiation in *Drosophila* embryonic development, Toll proteins were subsequently found to play important roles in antifungal defense alongside other antimicrobial peptides [1]. Mammalian homologs of Toll proteins were later discovered and designated as Toll-like receptors (TLRs) [2]. To date, at least 15 TLRs have been identified, with TLR1–TLR9 being common to both humans and mice. TLR10 is not expressed in mice, while TLR11–TLR13 are mouse-specific [3]. TLR14 and TLR15 have been successively identified in mice and chickens, although some reports suggest TLR14 expression in both humans and mice. Recent studies have demonstrated that TLRs, as a critical class of pattern recognition receptors (PRRs), play essential roles in intestinal immune regulation. Under normal conditions, the intestinal system maintains a complex and balanced state through intestinal mucosal immunity, with intestinal epithelial cells playing a key role in establishing this homeostasis. Concurrently, TLRs are vital for the intestinal immune system' s ability to discriminate between pathogenic and commensal bacteria [4]. Research on TLRs has become a major focus in life sciences, with deepening investigations into their ligands, signaling pathways, and biological functions, particularly regarding their influence on intestinal mucosal immune regulation.

1. TLR Structure and Ligands

The structural characteristics of TLRs determine their unique functions. TLRs consist of three domains: an extracellular region, a transmembrane region, and a cytoplasmic region. The extracellular domain contains leucine-rich repeats (LRRs), also known as the LRR functional domain, which recognizes and binds pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). The transmembrane region is rich in cysteine residues. The cytoplasmic Toll/interleukin-1 receptor (TIR) domain is considered the core region of TLRs, responsible for recruiting adaptor proteins containing TIR domains (TIR domain-containing adaptor protein, TIRAP) in the cytoplasm to initiate downstream signaling cascades [5]. These pathways ultimately lead to cytokine and chemokine production, activation of antimicrobial peptides, maturation of antigen-presenting cells, and recruitment of adaptive immune responses.

The high conservation of the TIR domain enables different TLRs to mediate similar signaling pathways.

1.2.1 PAMPs

Early detection of infection is beneficial for effective pathogen defense. Even minute quantities of PAMPs, including lipopolysaccharide (LPS), lipopeptides, unmethylated DNA, and double-stranded RNA, can trigger robust inflammatory responses. Many PAMPs elicit pro-inflammatory reactions that cause TLRs to release inflammatory mediators such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). Consequently, PAMPs like LPS, lipopeptides, and unmethylated DNA are among the most potent inducers of TNF-α and IL-1β release both *in vivo* and *in vitro* [6]. Viral RNA and bacterial DNA are present in late endosomal lysosomes, which is why TLR3, TLR7, and TLR9 are localized within these cellular organelles [7]. In contrast, TLR1, TLR2, TLR4, TLR5, and TLR6, which are positioned on the plasma membrane, recognize extracellular bacterial and fungal cell wall components as well as some viral proteins.

The compartmentalized recognition of TLR ligands to specific cellular locations such as the cell membrane or lysosomes not only increases the opportunity for TLRs to bind specific PAMPs but also reduces the possibility of aberrant TLR activation in the host. Therefore, an additional control level is necessary to ensure appropriate TLR activation.

1.2.2 DAMPs

Endogenous ligands refer to components derived from the host, including extracellular matrix degradation products and heat shock proteins released during tissue damage or physiological stress. These ligands can activate TLRs under both normal and altered environmental conditions. Since many endogenous ligands are associated with tissue injury, they are designated as DAMPs. Some TLRs can recognize multiple ligands, often with completely different structures. TLR2 and TLR4 are prime examples: TLR4 recognizes lipid components such as the lipid A portion of LPS, as well as proteins from respiratory syncytial virus, vesicular stomatitis virus, and mouse mammary tumor virus [8-10]. The ability of TLRs to recognize ligands independent of their chemical composition is thought to underlie their capacity to identify endogenous ligands. However, debate continues regarding whether DAMPs are indeed true TLR ligands. One hypothesis suggests that bacterial products or substances activated by TLRs, such as lipids or DNA, activate TLRs rather than the ligands themselves [11]. Future research will involve genetic inactivation of candidate molecules to determine whether DAMPs are authentic TLR ligands, and some of the aforementioned candidate factors may be confirmed as such. Additionally, endogenous DNA can act as a TLR9 agonist and promote autoimmune responses.

2.1 Signal Transduction

Binding of TLR agonists to their receptors initiates activation of a complex intracellular signaling network that coordinates inflammatory responses. Upon ligand binding, TLRs undergo conformational changes and dimerization. Key components of these signaling networks include adaptor proteins and several protein kinases such as extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p38 MAP kinase, phosphatidylinositol-3 kinase (PI-3K), and transcription factors IRF3/5/7, nuclear factor B (NF- B), and activator protein-1 (AP-1). Activation of these transcription factors leads to production of pro-inflammatory cytokines or co-stimulatory molecules involved in antiviral responses. Critical adaptor proteins include myeloid differentiation factor 88 (MyD88) and TIRAP, which are utilized by nearly all TLRs except TLR3, as well as TIR domain-containing adaptor inducing IFN-β (TRIF) and TRIF-related adaptor molecule (TRAM), both of which are important TIR-containing adaptor proteins. Among TLRs, only TLR4 can activate two distinct signaling pathways: TIRAP/MyD88 and TRAM/TRIF. Interestingly, TLR4 signaling via MyD88 does not require translocation from the plasma membrane to endocytic vesicles, whereas TRIF-mediated signaling requires TLR4 internalization [12]. The MyD88-dependent pathway activates downstream signaling components including IL-1 receptor-associated kinases (IRAKs), tumor necrosis factor receptor-associated factor 6 (TRAF6), transforming growth factor-activated kinase 1 (TAK1), and inhibitor of NF- B (I B). Activation of the MyD88-dependent pathway through NF- B and AP-1 induces production of pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12), and TNF-α. In contrast, the TRIF-dependent pathway activates interferon regulatory factors (IRFs) and induces type I interferon production (Figure 1 [Figure 1: see original paper] illustrates TLR signaling pathways).

Thus, both MyD88-dependent and -independent pathways are crucial for TLR-mediated regulation of immune responses.

HMGB1: high mobility group box-1 protein; **Hyaluronan:** hyaluronic acid; **ssRNA:** single-stranded ribonucleic acid; **TBK1:** tank-binding kinase 1; **IRAK4:** interleukin-1 receptor-associated kinase 4; **RIP1:** receptor-interacting protein 1; **IRF-3:** interferon regulating factor 3; **JNK:** c-Jun N-terminal kinase; **IFN-α/β:** interferon-α/β.

Figure 1. TLR signaling transduction pathway [13]. TLRs are primarily activated by viral PAMPs located in endosomes and bacterial PAMPs on the cell surface. In addition to PAMPs, several endogenous mediators including hyaluronan and high mobility group box-1 protein (HMGB1) can activate TLR2 and TLR4. TLRs induce upregulation of pro-inflammatory and antiviral genes through two adaptor molecules, MyD88 and TRIF. MyD88-mediated signaling (orange) primarily activates NF- B, IRF-7, and JNK, whereas TRIF-dependent signaling (blue) mainly activates NF- B and IRF-3.

2.2 Negative Regulation

Although robust pro-inflammatory and antiviral responses can effectively eliminate pathogens in the short term, chronic or excessive activation of TLR signaling can cause physiological dysfunction and lead to various diseases, including LPS-induced endotoxic shock, autoimmune disorders, and other TLR-related pathologies. It can also promote tumor development, metastasis, and immune evasion, particularly in inflammation-associated cancers [14]. Consequently, several mechanisms for negative regulation of TLR-induced intracellular responses have evolved [15]. These mechanisms operate at the receptor level [through expression of radioprotective 105 (RP105), suppression of tumorigenicity protein 2 (ST2), and single immunoglobulin IL-1 receptor-related protein (SIGIRR), as well as TLR downregulation or degradation] and at the adaptor level [targeting molecules such as MyD88 and TIRAP or corresponding kinases like IRAK]. These regulatory mechanisms reduce TLR signaling through decreased TLR4 transcription, degradation of TLR4 and TLR9 proteins, TIRAP degradation by inhibitory zinc finger protein Triad3A or suppressor of cytokine signaling 1 (SOCS-1), inhibition of TIR domains in ST2 and SIGIRR, suppression of LRR domain receptors such as RP105, and expression of non-functional signaling molecules including short splice variants of MyD88, IRAK-M, IRAK2c, and IRAK2d.

3.1 TLRs in the Intestine

Most TLRs are expressed in the animal intestinal tract, though this section focuses on only partial expression patterns. TLR5 is primarily expressed on colonic epithelial cells and recognizes invasive flagellated bacteria, whereas TLR2 and TLR4 show low expression in intestinal epithelium but are more abundant in colonic crypts [16]. TLR3 is mainly expressed in mature intestinal cells of the small intestine and colon [17-18]. The differential spatial distribution of TLRs within epithelial cells may constitute a key regulatory mechanism for discriminating between commensal and pathogenic bacteria.

3.2 TLRs Linking Innate and Adaptive Immunity

Invading pathogens are controlled by both innate and adaptive immunity within the immune system, eliciting systemic and mucosal immune responses. The innate immune system serves as the first line of defense against pathogen invasion and plays a critical role in early pathogen recognition and subsequent initiation of inflammatory responses [6]. Innate immune responses depend on TLR recognition of relevant molecular patterns. When pathogens emerge, TLRs transmit danger signals to antigen-presenting cells, while ligand-TLR binding induces production of reactive oxygen species, nitrogen dioxide, and pro-inflammatory factors, and upregulates co-stimulatory molecule expression, ultimately triggering adaptive immunity.

The intestine must control microbial populations and identify potential

pathogens—tasks accomplished collectively by the epithelial barrier, innate immunity, and adaptive immune mechanisms (Figure 2 [Figure 2: see original paper]). Adaptive immunity establishment involves cell proliferation, gene activation, and protein synthesis, making pathogen elimination often insufficiently rapid. Innate immune responses provide a more rapid defense mechanism through PRR recognition of invading pathogens. TLRs can activate innate immunity and mount primary inflammatory responses before adaptive immunity develops.

MAMPS: microbe-associated molecular patterns; **NLR:** nod-like receptors; **Defensins:** defensins; **Innate lymphoid cell:** innate lymphoid cell; **Goblet cell:** goblet cell; **Paneth cell:** Paneth cell; **Stromal cell:** stromal cell; **Macrophage:** macrophage; **Dendritic cell:** dendritic cell.

Figure 2. Mechanism of intestinal response against MAMPs and DAMPs under normal conditions [19]. The epithelial barrier recognizes microbial-associated molecular patterns (MAMPs) through transmembrane TLRs, while intracellular microorganisms and DAMPs are recognized by nod-like receptors (NLRs). Pathogens invading the lamina propria are recognized by other cells such as dendritic cells, macrophages, and lymphocytes through the same mechanisms, and the resulting chemokines and cytokines activate immune cells within the lamina propria. TLRs and NLRs initiate innate immunity and maintain homeostasis.

3.3 TLRs Activating Intestinal Epithelial Cells

Animal health depends on the intestinal system as the primary health barrier [20], a function accomplished through both intestinal microbiota structure and epithelial cell health status. First, microbial flora in the intestine forms a mechanical barrier against pathogens through close association with intestinal epithelial cells. Additionally, intestinal epithelial cells provide early signals for initiating and regulating inflammatory responses and secrete antimicrobial peptides [21] to maintain intestinal health. To establish a rich, long-term homeostatic relationship between the host and microbiota, TLRs actively participate in creating the intestinal environment by converting pathogen-associated molecular patterns into signals for antimicrobial peptide expression, barrier reinforcement, and epithelial cell proliferation. Intact TLR signaling is required for healing damaged intestinal epithelium and clearing bacteria within the mucosa. Functional impairment of epithelial TLRs is associated with chronic diseases such as Crohn' s disease and cancer.

Antibiotic therapy disrupts intestinal microbiota homeostasis and compromises intestinal immune defense, while also promoting resistance gene transfer by inducing prophages in gut microbes. As alternatives to antibiotics, antimicrobial peptides exert positive effects on animal intestinal health. The process of antimicrobial peptide secretion regulated by TLRs is consequently complex. Paneth cells (PCs), located at the base of small intestinal crypts, regulate intestinal microbiota composition and resist pathogens through effector molecules such as de-

fensins. Intestinal defensins are endogenous antimicrobial peptides whose transcriptional induction and secretion require TLR regulation. In addition to broad-spectrum antimicrobial activity, defensins can modulate immune responses. Reduced epithelial defensins may cause intestinal bacterial translocation in animals. Studies have shown that porcine β-defensin 1 (pBD1) and porcine β-defensin 2 (pBD2) affect mucin and tight junction protein gene expression in porcine intestinal epithelial cells [22]. Paneth cells express α-defensins, defensin-5, and defensin-6, and intact bacteria or PAMPs such as LPS, lipoteichoic acid (LTA), and muramyl dipeptide can stimulate defensin release, indicating that Paneth cells express a broad range of TLRs. Paneth cells express TLR9 in their secretory granules, and widespread induction of Paneth cell degranulation releases antimicrobial peptides to protect mice against *Salmonella typhimurium* infection [23]. Besides defensins, Hopper et al. [24] have demonstrated that luminal bacteria induce angiogenin expression in Paneth cells. These findings indicate that Paneth cell antimicrobial functions are TLR-associated.

In addition to Paneth cell expression of α-defensins, intestinal epithelial cells can also express α-defensin 1, α-defensin 2, and α-defensin 3 [25]. Researchers have confirmed that *Salmonella* flagellin stimulates β-defensin 2 expression in intestinal epithelial cells [26-27], and TLR4- and TLR2-dependent signaling pathways can stimulate β-defensin 2 expression in intestinal epithelial cells [28]. These data demonstrate that intestinal epithelial cells can combat pathogens and maintain intestinal health by secreting antimicrobial peptides under TLR regulation.

3.4 TLR Interaction with Intestinal Commensal Bacteria

While intestinal microorganisms can pose a threat to the intestinal mucosal barrier as antigens, commensal bacteria also maintain intestinal mucosal barrier homeostasis by activating the intestinal immune system [29]. Rakoff-Nahoum et al. [30] demonstrated that commensal bacteria play a positive role in preventing tissue damage and intestinal injury through TLR activation. Commensal bacteria can also resist pathogen infection by producing metabolites or secreting antimicrobial substances, with antimicrobial peptides shaping microbial communities in the intestine [31]. Antimicrobial peptides in the rumen of ruminants can alter rumen microbial composition, regulate rumen fermentation, and reduce associated diseases [32]. Wu et al. [33] showed that feeding antimicrobial peptides to pigs inhibited harmful bacteria proliferation in the cecum while stimulating beneficial bacteria growth. Various *Bifidobacterium* species (commensal bacteria) can produce organic acids and polypeptides [34] that affect pathogenic *E. coli* adhesion to endothelial cells, which is fundamental for pathogen colonization and virulence [35]. Pathogens adhering to mucosal surfaces and/or invading tissues can be detected by TLRs, which initiate appropriate responses. During *Clostridium difficile* infection, intestinal commensal bacteria can activate TLR signaling pathways [36], promoting colonic lamina propria cell expression of chemokine CXCL1 to resist pathogens.

The discovery of TLRs represents a milestone in immunology, and in-depth research on these receptors has profound implications for both immunological theory advancement and disease treatment. Beyond their role in innate immune responses, TLRs are also important in many other processes, including adaptive immunity, regulation of sterile inflammation, wound healing, promotion of epithelial regeneration, and carcinogenesis. Currently, research on the key role of TLRs in intestinal mucosal immune regulation has focused primarily on humans, and the full spectrum of TLR effects on intestinal and animal tissue health remains incompletely understood. Therefore, substantial research is still needed on TLR impacts on animal intestinal health, such as investigating TLR expression levels in different tissues and organs of animals at various ages and production states, particularly young animals whose intestinal health remains vulnerable from birth to weaning. Additional research should examine correlations between nutritional interventions or different exogenous immune agents (such as antimicrobial peptides) and their effects on animal intestinal microbiota with upregulation or downregulation of TLRs, as well as relationships between TLR changes and other immune factor profiles. As related research progresses, it will provide theoretical foundations for immunotherapeutic approaches using antibiotic alternatives for infectious diseases, contributing to human health and sustainable livestock development.

References

[1] AKIRA S, TAKEDA K. Toll-like receptor signalling [J]. Nature Review Immunology, 2004, 4(7): 499-511.
[2] AKIRA S, TAKEDA K, KAISHO T. Toll-like receptors: critical proteins linking innate and acquired immunity[J]. Nature Immunology, 2001, 2(8): 675-680.
[3] THOMPSON A J V, LOCARNINI S A. Toll-like receptors, RIG-I-like RNA helicases and the antiviral innate immune response[J]. Immunol Cell Biology, 2007, 85(6): 435-445.
[4] LUO J, LI W, DUAN Y F, et al. Host discriminates between probiotics and pathogens: impact of receptor 5-flagellin interaction evolution[J]. Microbiology China, 2014, 41(7): 1368-1375.
[5] KAWAI T, AKIRA S. The roles of TLRs, RLRs and NLRs in pathogen recognition[J]. Int Immunol, 2009, 21(4): 317-337.
[6] BEUTLER B A. TLRs and innate immunity[J]. Blood, 2009, 113(7): 1399-1407.
[7] AKIRA S, UEMATSU S, TAKEUCHI O. Pathogen recognition and innate immunity[J]. Cell 2006, 124(4): 783-801.
[8] GEORGEL P, JIANG Z F, KUNZ S, et al. Vesicular stomatitis virus glycoprotein G activates a specific antiviral toll-like receptor 4-dependent pathway[J]. Virology, 2007, 362(2): 304-313.
[9] JUDE B A, POBEZINSKAYA Y, BISHOP J, et al. Subversion of the innate immune system by a retrovirus[J]. Nature immunology, 2003, 4(6): 573-578.
[10] KURT-JONES E A, POPOVA L, KWINN L, et al. Pattern recognition

receptors TLR4 and CD14 mediate response to respiratory syncytial virus[J]. Nature Immunology, 2000, 1(5): 398-401.
[11] TSAN M F, GAO B. Pathogen-associated molecular pattern contamination as putative endogenous ligands of Toll-like receptors[J]. Journal of Endotoxin Research, 2007, 13(1): 6-14.
[12] KAGAN J C, SU T, HORNG T, et al. TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-β[J]. Nature Immunology, 2008, 9(4): 361-368.
[13] MENCIN A, KLUWE J, SCHWABE R F. Toll-like receptors as targets in chronic liver diseases[J]. Gut, 2009, 58(5): 704-720.
[14] CHENG Y X, QI X Y, HUANG J L, et al. Toll-like receptor 4 signaling promotes the immunosuppressive cytokine production of human cervical cancer[J]. European Journal of Gynaecological Oncology, 2012, 33(3): 291-294.
[15] LIEW F Y, XU D, BRINT E K, et al. Negative regulation of toll-like receptor-mediated immune responses[J]. Nature Reviews Immunology, 2005, 5(6): 446-458.
[16] WELLS J M, LOONEN L M P, KARCZEWSKI J M. The role of innate signaling in the homeostasis of tolerance and immunity in the intestine[J]. Int J Med Microbiol, 2010, 300(1): 41-48.
[17] WELLS J M, ROSSI O, MEIJERINK M, et al. Epithelial crosstalk at the microbiota-mucosal interface[J]. Proceedings of the National Academy of Sciences of the United States of America, 2011, 108(Suppl.1): 4607-4614.
[18] CARIO E, ROSENBERG I M, BRANDWEIN S L, et al. Lipopolysaccharide activates distinct signaling pathways intestinal epithelial cell lines expressing toll-like receptors[J]. Journal of Immunology, 2000, 164(2): 966-972.
[19] ELIA P P, TOLENTINO Y E, BERNARDAZZI C, et al. The role of innate immunity receptors pathogenesis inflammatory bowel disease[J]. Mediators Inflammation, 2015, 2015: 936193.
[20] TEIXEIRA L D, SILVA O N, MIGLIOLO L, et al. In vivo antimicrobial evaluation of an alanine-rich peptide derived from *Pleuronectes americanus*[J]. Peptides, 2013, 42: 144-148.
[21] ZENG Jiong, HUANG Xingguo, WU Liyang. Research progress on the relationship between microecological preparations and intestinal mucosal immunity[J]. Feed Industry, 2010, 31(4): 58-60.
[22] XUE Xianfeng. Antimicrobial and antioxidant functions of porcine β-defensins and their effects on mucosal barrier function of intestinal epithelial cells[D]. Master' s thesis, Hangzhou: Zhejiang University, 2012.
[23] RUMIO C, BESUSSO D, PALAZZO M, et al. Degranulation of paneth cells via toll-like receptor 9 [J]. The American Journal of Pathology, 2004, 165(2): 373-381.
[24] HOOPER L V, STAPPENBECK T S, HONG C, et al. Angiogenins: a new class of microbicidal proteins involved in innate immunity[J]. Nature Immunology, 2003, 4: 269-273.
[25] FAHLGREN A, HAMMARSTRÖM S, DANIELSSON Å, et al. Increased expression of antimicrobial peptides and lysozyme in colonic epithelial cells of patients with ulcerative colitis[J]. Clinical & Experimental Immunology, 2003,

131(1): 90–101.
[26] OGUSHI, WADA A, NIIDOME T, et al. *Salmonella enteritidis* FliC (flagella filament protein) induces human β-defensin-2 mRNA production by Caco-2 cells[J]. Journal of Biological Chemistry, 2001, 276(32): 30521–30526.
[27] OGUSHI K, WADA A, NIIDOME T, et al. Gangliosides act as co-receptors for *Salmonella enteritidis* FliC and promote FliC induction of human beta-defensin-2 expression in Caco-2 cells[J]. Journal of Biological Chemistry, 2004, 279(13): 12213–12219.
[28] VORA P, YOUDIM A, THOMAS L S, et al. β-defensin-2 expression is regulated by TLR signaling in intestinal epithelial cells[J]. Journal of Immunology, 2004, 173(9): 5398–5405.
[29] ZUO Zengyan, ZHANG Cai. Research progress on intestinal mucosal immune tolerance mechanisms[J]. Modern Immunology, 2015, 35(1): 68–71.
[30] RAKOFF-NAHOUM S, PAGLINO J, ESLAMI-VARZANEH F, et al. Recognition of commensal microflora by toll-Like receptors required intestinal homeostasis [J]. Cell, 2004, 118(2): 229–241.
[31] CAI Jie, TANG Zhiru, DENG Huan, et al. Research progress on the mechanism of antimicrobial peptides in animal intestinal mucosa maintaining microbial flora balance[J]. Journal of Animal Nutrition, 2014, 26(8): 2071–2076.
[32] CHEEMA U B, YOUNAS M, SULTAN J I, et al. Antimicrobial peptides: an alternative of antibiotics in ruminants[J]. Advances in Agricultural Biotechnology, 2011(2): 15–21.
[33] WU S D, ZHANG F R, HUANG Z M, et al. Effects of the antimicrobial peptide cecropin AD on performance and intestinal health in weaned piglets challenged with *Escherichia coli*[J]. Peptides, 2012, 35(2): 225–230.
[34] SCHOSTER A, KOKOTOVIC B, PERMIN A, et al. In vitro inhibition of *Clostridium difficile* and *Clostridium perfringens* by commercial probiotic strains[J]. Anaerobe, 2013, 20: 36–41.
[35] TANG Zhigang, WEN Chao, WANG Tian, et al. Structure-activity relationship between probiotics and poultry intestinal immune regulation[J]. Animal Husbandry and Veterinary Medicine, 2012, 44(8): 86–89.
[36] BUFFIE C G, PAMER E G. Microbiota-mediated colonization resistance against intestinal pathogens[J]. Nature Reviews Immunology, 2013, 13(11): 790–801.

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