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Immune Tolerance Mechanisms of the Gut to Commensal Microbiota (Post-print)

Authors: Yue Jiaqing, Wang Jiakun

Date: 2017-10-23T00:00:00+00:00

Abstract

Trillions of microorganisms inhabit the intestinal tract of animal hosts. These symbiotic microbes assist in host digestion and metabolism and maintain intestinal homeostasis. However, microorganisms and their metabolic products can also serve as antigens that influence normal intestinal function. Under normal conditions, the intestinal immune system can accurately recognize symbiotic microorganisms and their metabolic products, develop immune tolerance toward them, and maintain internal homeostasis. Furthermore, the intestinal immune system can avoid the waste of immune resources resulting from responses to harmless antigens. Immune tolerance has been widely applied in clinical medicine to reduce rejection following organ transplantation, decrease maternal immune rejection of the fetus, etc. However, few reports exist on utilizing the immune tolerance mechanism to alleviate rumen acidosis in ruminants, improve the synthesis and utilization efficiency of rumen microbial protein, and optimize probiotic feeding protocols. Therefore, this review elucidates the general concept and applications of immune tolerance, the composition and function of the intestinal immune system, the immunogenicity of intestinal symbiotic microorganisms, and the mechanisms underlying intestinal immune tolerance formation.

Full Text

Intestinal Immune Tolerance Mechanism of Symbiotic Microorganisms

Le Jiaqing, Wang Jiakun*
Institute of Dairy Science, Zhejiang University, Hangzhou 310058, China

Abstract

The animal gut harbors hundreds of millions of microorganisms that assist in digestion, metabolism, and maintenance of intestinal homeostasis. However, these microorganisms and their metabolites can also function as antigens that affect normal intestinal function. Under normal conditions, the intestinal immune system accurately identifies symbiotic microorganisms and their metabolites, mounting an immune tolerance response that maintains internal environmental stability while avoiding wasteful immune reactions to harmless antigens. Although immune tolerance has been widely applied in clinical medicine to reduce organ transplant rejection and decrease maternal immune rejection of the fetus, few studies have reported on utilizing immune tolerance mechanisms to alleviate rumen acidosis in ruminants, improve rumen microbial protein synthesis and utilization efficiency, or refine probiotic feeding protocols. This review therefore elaborates on the general concepts and applications of immune tolerance, the composition and function of the intestinal immune system, the immunogenicity of intestinal symbiotic microorganisms, and the mechanisms underlying intestinal immune tolerance.

Keywords: intestinal symbiotic microorganisms; intestinal immune system; intestinal immune tolerance; mechanism

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Microorganisms constitute an indispensable component of the animal digestive tract. Through long-term evolution, animals and microorganisms have established a relationship of mutualistic symbiosis and mutual constraint. Microorganisms assist in host digestion and metabolism, inhibit pathogen colonization, and maintain digestive tract homeostasis. Simultaneously, microbial components such as bacterial DNA and metabolic products like endotoxin contain numerous immune stimulants that can affect normal digestive function, and these immune stimulants can even cross the digestive barrier to infect other organs and cause lesions. As the site most extensively exposed to environmental antigens, the digestive tract must normally mount immune tolerance to harmless antigens such as food and symbiotic microorganisms—defined as a state of non-responsiveness when immunologically active cells encounter antigenic substances—thereby avoiding wasteful immune resource expenditure. Conversely, it must also generate immune rejection and clearance of pathogens. Understanding how the intestine accurately identifies different substances and produces these two distinct immune responses can help improve probiotic application strategies and selectively enhance rumen microbial protein composition in ruminants while reducing immune consumption. This article therefore reviews the general concepts and applications of immune tolerance, the composition and function of the intestinal immune system, the immunogenicity of intestinal symbiotic microorganisms, and the mechanisms of intestinal immune tolerance.

Received: 2016-11-04
Funding: Fundamental Research Funds for the Central Universities

(2015FZA6016)
Author: Le Jiaqing (1992-), female, master' s student, engaged in ruminant nutrition research. E-mail: 945841362@qq.com
*__Corresponding author:__ Professor, doctoral supervisor, E-mail: jiakunwang@zju.edu.cn

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1. General Concepts and Applications of Immune Tolerance

Immune tolerance refers to the phenomenon where antigen-specific T and B cells, upon antigen stimulation, fail to be activated, cannot produce specific immune effector cells and antibodies, and thus cannot execute specific immune responses. Antigens that induce immune tolerance are called tolerogens. Immune tolerance is divided into natural immune tolerance and acquired immune tolerance. Self-tissue antigens can induce natural immune tolerance, while non-self antigens such as pathogenic microorganisms can become tolerogens and induce acquired immune tolerance under certain conditions. Immune tolerance can also be artificially induced by simulating natural immune processes.

Due to its memory characteristics, when an organism already tolerant to a specific antigen encounters the same antigen again, it produces a specific non-responsive reaction, while retaining responsiveness to other antigens. This property has been widely applied in clinical medicine. Before organ transplantation, inducing immune tolerance to donor antigens can prevent post-transplant rejection. Scandling et al. performed HLA-matched kidney transplantation combined with donor bone marrow transplantation, with results showing over half of patients no longer required conventional immunosuppressants. Correale et al. found that helminth infection in multiple sclerosis (MS) patients alleviated MS symptoms, which recurred after anti-helminth treatment. Immune tolerance also plays important roles in preventing spontaneous abortion and intrauterine growth restriction. Arck et al. demonstrated that during early pregnancy, fetal extravillous trophoblast (EVT) cells invade decidual tissue, directly contacting maternal decidual immune cells (DIC) to establish a sophisticated maternal-fetal dialogue that promotes a maternal-fetal immune tolerance microenvironment for normal fetal development. Immune tolerance is equally important in animal husbandry. Wang et al. found that oral progesterone administration during dairy cow pregnancy induced increased regulatory T cells in peripheral blood, thereby reducing uterine immune rejection of the fetus. The superior efficacy of probiotics in young versus adult animals also involves immune tolerance mechanisms.

2. Composition and Function of the Intestinal Immune System

The intestinal immune system, part of the overall immune system, is widely distributed in the intestinal mucosa and directly contacts environmental antigens. It plays crucial roles not only in identifying and clearing pathogens but also in forming immune tolerance to beneficial substances such as food and symbiotic microorganisms. The intestinal immune system is primarily composed of gut-associated lymphoid tissue (GALT), which can be divided into two components based on morphology and function. First, GALT consisting of mucosal lymph nodes, mainly including Peyer' s patches (PP) and mesenteric lymph nodes (MLN), serves as the induction site for immune responses. Second, GALT composed of leukocytes widely distributed in the mucosal epithelium and lamina propria, mainly including lamina propria mononuclear cells and intraepithelial lymphocytes (IEL), functions as the effector site for immune responses.

Peyer' s patches are located in the submucosa of the small intestine and contain abundant B cells, T cells, macrophages (Mø cells), and dendritic cells (DC cells). They are separated from the intestinal lumen by follicle-associated epithelium (FAE). Unlike mucosal epithelial cells, FAE has lower digestive enzyme content, an inconspicuous brush border, and lacks receptors for polymeric immunoglobulin A (IgA). The most distinctive feature of FAE is the presence of microfold (M) cells, which exhibit high transcellular transport activity and are generally considered the initiation site of the intestinal immune system.

MLN are the largest lymph nodes in the body. Unlike Peyer' s patches, their development is not affected by most cytokines such as tumor necrosis factor (TNF) and its receptors. Current research suggests MLN may serve as a crossroads between the external environment and the mucosal recirculation pathway.

The lamina propria is infiltrated by numerous lymphocytes and myeloid cells, including T cells, B cells, Mø cells, neutrophils, other granulocytes, and mast cells, all playing important roles in regulating immune responses.

IEL are primarily CD3+ T cells, also including secretory IgA (SIgA)+ B cells and natural killer (NK) cells. These cells eliminate invading pathogens and degenerated cells through perforin, granzyme, and Fas receptors. Additionally, they regulate other lymphocytes and epithelial cells by secreting interleukin (IL)-22, TNF-2 , and transforming growth factor- (TGF-) to achieve immune function. Lamina propria lymphocytes (LPL) mainly comprise CD4+ T cells and SIgA+ B cells. T cells primarily regulate immune responses by secreting IL-10 and TGF- to downregulate immune reactions and can also influence B cell production of SIgA. B cells perform immune functions by secreting SIgA, which specifically binds antigens to prevent invasion and destruction of the mucosal barrier.

3. Immunogenicity of Intestinal Symbiotic Microorganisms

The animal gut contains numerous microorganisms that reside in the intestine, forming a relationship of mutualistic symbiosis and mutual constraint with the host. These symbiotic microorganisms can act as antigens to stimulate specific immune cells in the intestine, causing activation, proliferation, and differentiation that ultimately produces specific antibodies or sensitized lymphocytes to induce immune responses. This property of inducing immune responses is called immunogenicity. Symbiotic microorganisms and their metabolites contain abundant immunogenic substances. First, symbiotic microorganisms contain substantial nucleic acids, which are mostly non-immunogenic but become immunogenic when combined with proteins to form nucleoproteins. Second, the massive bacterial cell walls of symbiotic microorganisms contain components such as peptidoglycan, teichoic acid, and lipopolysaccharide (LPS), some of which are immunogenic. Studies have shown that LPS, a cell wall component of Gram-negative bacteria, functions as an endotoxin with strong immunogenicity that can cause immune reactions and inflammatory diseases when acting in large quantities on intestinal tissues. Additionally, large molecular proteins, polysaccharides, and small molecular peptides produced by symbiotic microorganisms through digestion and metabolism are also immunogenic.

However, since the establishment of gut microbiota is largely synchronized with immune system maturation, animals are born sterile but become colonized by microorganisms within a very short time after birth. Due to the fragile regulatory network of the neonatal immune system, contact with microbial antigens easily leads to lifelong or long-term tolerance—specific non-responsive reactions to microorganisms. Under normal conditions, due to intestinal immune tolerance to symbiotic microorganisms, the large number of symbiotic microbes in the gut does not cause excessive immune responses. Therefore, studying and understanding the mechanisms of intestinal immune tolerance to symbiotic microorganisms has important application value for disease prevention and treatment in medicine and animal nutrition.

4. Mechanisms of Intestinal Immune Tolerance to Symbiotic Microorganisms

Intestinal immune tolerance mechanisms to symbiotic microorganisms include both natural and acquired immune tolerance. Natural immune tolerance can occur when the intestine lacks receptors for certain microbial antigens or when inhibitory receptors and structures exist on intestinal cell surfaces. Current research has identified important roles for Mø cells in Peyer' s patches in forming natural immune tolerance to symbiotic microorganisms.

Acquired immune tolerance can be established when mature T and B cells in the intestine encounter symbiotic microbial antigens. Multiple types of acquired immune tolerance exist. First, activation of mature T or B cells requires two or more signals; when some signaling factors are inhibited, T or B cells cannot

be activated and remain unresponsive, forming acquired immune tolerance. DC cells, Toll-like receptors (TLRs), and peroxisome proliferator-activated receptor (PPAR) are all involved in this type of tolerance formation. Second, when harmless antigens such as symbiotic microorganisms fail to contact immune cells due to certain reasons, they enter a state of neglect, thereby forming immune tolerance. The intrinsic characteristics of symbiotic microorganisms and intestinal alkaline phosphatase (ALPI) play important roles in this tolerance formation. Third, binding between FasL (CD178) and Fas (CD95) on T-B or T-T cells can initiate activation-induced cell death (AICD), or apoptosis, eliminating T or B cells reactive to harmless antigens such as symbiotic microorganisms. Expression of FasL and Fas is controlled by many factors, including interferons and ILs. Some studies suggest that decreased IL-2 and IL-12 levels enhance Fas-mediated apoptosis of antigen-specific T cells. Finally, immunoregulatory cells such as regulatory T cells (Treg cells) secrete inhibitory cytokines to form immune tolerance.

The following sections specifically elaborate on the roles of Mø cells, DC cells, TLRs, PPAR- , intrinsic microbial characteristics, ALPI, and Treg cells in intestinal immune tolerance to symbiotic microorganisms.

4.1. Mø Cell-Mediated Immune Tolerance Mechanisms

Mø cells, a type of leukocyte, phagocytose and digest cellular debris, microorganisms, cancer cells, and foreign substances, and are widely distributed in the intestine. Current research suggests at least two Mø cell subtypes exist: M1 classical Mø cells and M2 non-classical Mø cells. M1 Mø cells are activated by interferon- (IFN-) and LPS, participate in pro-inflammatory response regulation, and play important roles in host defense against pathogenic bacteria and viral infections. M1 Mø cells promote NF- B-dependent transcription of inflammatory chemokines, secrete inducible nitric oxide synthase (iNOS) and pro-inflammatory factors such as TNF and IL-6, and induce Th1 responses to clear pathogens. M2 Mø cells are activated by IL-4, IL-13, and immune complexes, and are further divided into M2a, M2b, and M2c subtypes based on activation mode. They participate in anti-inflammatory response regulation and are also associated with tissue reconstruction, fibrosis, and tumorigenesis. Activated M2 Mø cells do not produce pro-inflammatory factors such as TNF and IL-6 but mainly secrete the anti-inflammatory factor IL-10. Moreover, signals induced by M2 Mø cells inhibit chemokine production by M1 Mø cells. Mantovani et al. demonstrated that IL-4 and IL-10 can inhibit IFN- -dependent production of inflammatory chemokines CXCL10 and CCL5, while IL-10 can inhibit I B kinase (IKK) activity to suppress NF- B activation. Different Mø cell subtypes recognize different microorganisms, suggesting that intestinal symbiotic microorganisms may form immune tolerance through recognition by M2 Mø cells.

Second, Mø cells function through recognition via surface polysaccharide receptors, and some intestinal symbiotic microorganisms may evade macrophage

recognition due to lack of corresponding polysaccharides or because polysaccharides are covered by cell surface glycoproteins, thereby forming immune tolerance.

Furthermore, Smythies et al. showed that human intestinal Mø cells do not express certain receptors, including the LPS receptor CD14, the IgA Fc segment (Fc) receptor CD89, and the IL-2 receptor CD25. Additionally, intestinal Mø cells do not produce pro-inflammatory cytokines such as IL-1 and IL-6 but retain phagocytic and bactericidal functions when responding to various inflammatory stimuli.

4.2. DC Cell-Mediated Immune Tolerance Mechanisms

DC cells function as both antigen-presenting cells and regulators of intestinal mucosal immunity. Widely present in Peyer' s patches, they mainly comprise three subtypes: the first expresses CD11b molecules and secretes IL-10 when stimulated by CD40L or killed by Staphylococcus aureus; the second expresses CD8 molecules; and the third expresses neither CD11b nor CD8 molecules and is called double-negative DC cells. DC cells regulate the diversity of CD4+ helper T cells. After antigen contact, CD4+ helper T cells differentiate into various types, mainly including Th1 cells secreting IFN- , Th2 cells secreting IL-4 and IL-13, Th3 cells secreting TGF- , Tr-1 cells secreting IL-10, and Treg cells. Th3, Tr-1, and Treg cells can suppress immune responses and may play important roles in immune tolerance.

Different DC subtypes directly cause differential differentiation of CD4+ helper T cells. CD8 + DC cells secrete IL-12 and induce Th1 responses, whereas CD11b+ DC cells secrete IL-10 and induce CD4+ antigen-specific T cells to secrete large amounts of IL-10 and differentiate into Treg cells, thereby inducing immune tolerance.

Under normal conditions, most DC cells in the intestine are immature, expressing only low levels of major histocompatibility complex (MHC) class molecules while retaining strong antigen uptake and processing capacity. However, because immature DC cells essentially do not express CD40, CD80, and CD86 —co-stimulatory molecules required for T cell activation—they cannot activate T cells, forming a state of immune tolerance. Dumitriu et al. demonstrated that tumor cell stimulation could induce DC cells to secrete TGF- and inhibit expression of CD86 on mature DC cells, transforming them into immature DC cells. Such immature DC cells can induce naive CD4+ T cells to differentiate into Treg cells, promoting immune tolerance to tumor cells.

Therefore, intestinal symbiotic microorganisms may form immune tolerance by either promoting activation of immature DC cells with immune tolerance functions to indirectly influence CD4+ helper T cell differentiation, thereby suppressing activation of immune response-involved T or B cells, or by stimulating mature DC cells to transform into immature DC cells.

4.3. TLR-Mediated Immune Tolerance Mechanisms

Various cells in the intestinal mucosal epithelium, such as intestinal epithelial cells (IEC), DC cells, and Mø cells, widely express pattern recognition receptors known as the Toll-like receptor family (TLRs). The intestinal immune system relies on TLRs to recognize pathogen-associated molecular patterns (PAMPs) for substance identification. Different TLRs recognize different PAMPs for signal transduction: the TLR1/TLR2 complex recognizes triacyl lipopeptides, TLR2/TLR6 recognizes diacyl lipopeptides, TLR3 recognizes double-stranded RNA, the TLR4/MD-2 complex recognizes LPS, TLR5 recognizes flagellin, TLR7 recognizes imidazoquinoline, TLR8 recognizes single-stranded RNA, and TLR9 recognizes bacterial CpG DNA. After PAMP recognition, the intracellular domain of TLR binds to the C-terminal TIR (Toll/IL-1R) domain of the adaptor protein myeloid differentiation factor 88 (MyD88). MyD88 then binds to the N-terminus of IL-1 receptor-associated kinase (IRAK) to form a complex that triggers IRAK autophosphorylation. This subsequently induces oligomerization of the adaptor protein TNF receptor-associated factor-6 (TRAF-6), which activates mitogen-activated protein 3 kinase (MAP3K) family member transforming growth factor beta-activated kinase 1 (TAK1), leading to activation of IKK and IKK and phosphorylation and degradation of I B proteins. Ultimately, NF- B is released and translocates to the nucleus, where it induces expression of pro-inflammatory factors such as IL-1 and IL-8 together with other transcription factors to regulate immune responses. This is the classic MyD88-dependent TLR signaling pathway. MyD88-independent signaling pathways have also been identified, mainly mediated by TIRAP (also called Mal) and TRIF (also called TICAM-1), which bind to TLR2 or TLR4 and TLR3, respectively, to mediate NF- B signaling. Both MyD88-dependent and -independent pathways can induce CD80 and CD86 expression to trigger acquired immune responses.

However, recent research suggests that certain alterations in TLR-mediated signaling pathways can also mediate immune tolerance. For example, Luo et al. constructed phylogenetic trees of flagellins from intestinal probiotics and pathogens and compared TLR5 recognition sequences, finding that TLRs recognize different sites on symbiotic versus pathogenic microorganisms. The different flagellin recognition regions of pathogenic and probiotic bacteria may represent an adaptation to TLR5 recognition, enabling the host to distinguish pathogens from probiotics. Consequently, TLRs may fail to recognize some symbiotic microorganisms, preventing NF- B pathway activation and indirectly suppressing activation of immune response-involved T and B cells to form immune tolerance.

Additional studies show that activation of certain TLRs on the surface of intestinal epithelial cells inhibits I B degradation, thereby suppressing the NF- B pathway and indirectly inhibiting activation of immune response-involved T and B cells to form immune tolerance, whereas activation of certain basolateral TLRs promotes I B degradation, thereby activating the NF- B pathway and promot-

ing immune responses. Jongdae et al. demonstrated through NF- B activation and cDNA microarray analysis that apical and basolateral TLR9 in intestinal epithelial cells have different functions that are important for maintaining intestinal homeostasis. Correspondingly, some studies suggest that IECs lack certain TLRs, such as TLR4. Naik et al. compared TLR2 and TLR4 expression in human peripheral blood mononuclear cells and human intestinal epithelial cells, finding that TLR2 mRNA was highly expressed in peripheral blood mononuclear cells and also expressed in intestinal epithelial cells, whereas TLR4 was expressed only in peripheral blood mononuclear cells. They suggested that TLR4 deficiency is related to the low responsiveness of intestinal epithelial cells to LPS and that although TLR2 exists in intestinal epithelial cells, it is activated only when bacterial cell wall components reach pathologically high concentrations in the intestine.

4.4. PPAR- -Mediated Immune Tolerance Mechanisms

Peroxisomes in the body can remove molecular oxygen and hydrogen peroxide and are involved in glycolipid, bile acid, and cholesterol synthesis and fatty acid oxidation. Fatty acid-like chemicals that stimulate peroxisome proliferation are called peroxisome proliferators (PP). The receptors activated by peroxisome proliferators are PPARs, which exist in three subtypes across species: PPAR- , PPAR- / , and PPAR- . PPAR- regulates peroxisome proliferator gene transcription and hepatic peroxisome hyperplasia, while PPAR- regulates fatty acid metabolism in skeletal muscle and brown adipose tissue. PPAR- has been most extensively studied and is mainly expressed in adipose tissue, macrophages, and the large intestine. Interaction between PPAR- and its ligand 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) can regulate immune responses. Studies show that PPAR- binding to 15d-PGJ2 inhibits LPS-induced transcription pathways mediated by activator protein-1 (AP-1), NF- B, and signal transducer and activator of transcription 1 (STAT1). Through protein-protein interaction, PPAR- inhibits NF- B binding to homologous cis-elements in inflammatory factor gene promoter regions, suppressing the NF- B pathway and indirectly inhibiting activation of immune response-involved T and B cells to form immune tolerance. Knethen et al. found that 15d-PGJ2 and glitazones inhibit PHA-induced T cell proliferation and IL-2 gene expression by activating PPAR- , thereby preventing activated T cells from binding to homologous cis-elements in the IL-2 promoter. Activated PPAR- can inhibit production of pro-inflammatory factors TNF- , IL-1, IL-2, and IL-6 through a series of signal transduction events, exerting anti-inflammatory effects. Kelly et al. reported that *Bacteroides thetaiotaomicron* can trigger PPAR- binding to the REL-A subunit of the NF- B transcription complex, forming a complex that transports from nucleus to cytoplasm, thereby inhibiting transcription of NF- B-activated pro-inflammatory factors. Lai et al. showed that LPS stimulation increased mRNA expression of IL-1, IL-6, and TNF- in weaned piglets while also significantly increasing PPAR- mRNA expression. These recent findings indicate that symbiotic microorganisms can achieve immune tolerance by inducing PPAR- activation to inhibit NF- B ac-

tivity and indirectly suppress activation of immune response-involved T and B cells.

4.5. Mechanisms Involving Intrinsic Characteristics of Symbiotic Microorganisms

Symbiotic microorganisms differ significantly from pathogenic bacteria. First, most symbiotic microorganisms cannot express adhesion and invasion factors, preventing them from invading the intestinal mucus layer. Additionally, small intestinal peristalsis can flush symbiotic microorganisms away from the intestinal surface, preventing their adhesion to intestinal epithelium and destruction of the epithelial barrier, thereby weakening their ability to colonize the intestinal wall. Second, the intestinal mucus layer is composed of mucins whose binding sites compete with those on intestinal epithelial cells, preventing microbial adhesion to the epithelium and promoting clearance of microorganisms in the mucus layer during intestinal peristalsis. Pathogenic bacteria can secrete mucinases to degrade mucins and destroy the intestinal mucus layer, whereas some symbiotic microorganisms not only do not degrade mucins but can also promote mucin secretion, enhance intestinal epithelial tight junction function, and inhibit adhesion of certain pathogenic bacteria. Finally, studies have identified endotoxin as the main component of Gram-negative bacterial cell walls, essentially LPS, which is composed of O-specific chains, outer core, inner core, and lipid A—the biologically active component of LPS. Golenbock et al. suggested that some symbiotic microorganisms possess pentacylated lipid A, whereas pathogenic bacteria have hexacylated lipid A, which may explain the low endotoxin toxicity of symbiotic microorganisms and their inability to trigger intense inflammatory responses. These intrinsic characteristics of symbiotic microorganisms can prevent their contact with immune cells, thereby inducing immune tolerance.

4.6. ALPI-Mediated Immune Tolerance Mechanisms

Alkaline phosphatase (ALP) is an enzyme widely distributed in tissues such as liver, intestine, and placenta that is excreted from the liver into bile. This enzyme removes phosphate groups from the 5' ends of nucleic acid molecules through catalysis, converting DNA or RNA fragments from 5' -P ends to 5' -OH ends. ALP is not a single enzyme but a group of isoenzymes present in various animals including cattle, sheep, and mice. ALPI is an ALP derived from intestinal villous epithelium and fibroblasts. ALPI can act on LPS in Gram-negative bacterial cell walls, removing phosphate groups from LPS to inhibit LPS-induced inflammatory responses. Sayeda et al. infected mice with or without bovine intestinal ALP supplementation with *Salmonella typhimurium* and *Clostridium difficile*, finding that ALP-supplemented mice showed significantly reduced colonization by these pathogens and suppressed inflammatory responses. ALPI can degrade antigens from symbiotic microorganisms such as LPS, preventing such antigens from contacting immune cells and thereby inducing immune tolerance.

4.7. Treg Cell-Mediated Immune Tolerance Mechanisms

Treg cells, or suppressor T cells, are also called CD4+CD25+, CD4+CD25high, or CD4+CD25highFOXP3+ T cells due to their high expression of the forkhead transcription factor (FOXP3). Compared with other helper T cells, Treg cells have a clear differentiation advantage in the intestinal immune system and play important roles in immune tolerance. They mainly regulate antigen-presenting cell function through cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), CD39, and lymphocyte activation gene 3 (LAG-3). Interfering with Treg cells can trigger rheumatoid arthritis, allergic inflammation, inflammatory bowel disease, and type I diabetes mediated by Th1, Th2, and Th17 cells. Qureshi et al. showed that CTLA-4 contains two ligands (CD80 and CD86) and a reactive receptor CD28. Treg cells bind to CD80 and CD86 on DC cell surfaces, enabling CTLA-4 to capture these two ligands from opposing cells through trans-endocytosis. After transfer, these co-stimulatory ligands are degraded and removed by CTLA-4-expressing cells, impairing T cell activation pathways induced by CD28 and thereby suppressing T cell activation. LAG-3 is a CD4-related transmembrane protein expressed by regulatory T cells that can bind to major histocompatibility complex class II (MHC II). Cross-linking of MHC II due to acrylonitrile competition triggers an inhibitory signaling pathway mediated by immunoreceptor tyrosine-based activation motif (ITAM), including IgG Fc segment receptor (Fc R) and extracellular regulated protein kinases (ERK)-mediated recruitment of protein tyrosine phosphatase-1 (SHP-1), thereby inhibiting DC cell maturation and immunostimulatory capacity and further suppressing T cell activation. Additionally, CD39, a cell surface-associated ectonucleotidase, can be used to purify Treg cells with strong suppressive function. CD4+CD39+ T cells can catalyze the cleavage of adenosine triphosphate (ATP) to adenosine monophosphate (AMP) and further cleave adenosine. Treg cells can produce inhibitory cytokines such as IL-10, TGF- , and IL-35 that suppress T cell function and lead to immune tolerance formation. Symbiotic microorganisms can promote proliferation and functional activity of CD4+Foxp3+ Treg cells in MLN and Peyer' s patches, leading to immune tolerance to symbiotic microorganisms. For example, Young et al. transferred CD4+VEGFR1high T cells into RAG2 knockout mice and ameliorated inflammatory bowel disease caused by lymphocyte deficiency. Bae et al. found that baicalein could induce Treg cell differentiation through the aryl hydrocarbon receptor, upregulate Treg cell-related factor expression, enhance intestinal barrier function, and thereby improve food allergy symptoms while reducing serum IgE and effector T cell activity. In summary, symbiotic microorganisms may form immune tolerance by promoting Treg cell activation and functional activity.

Although this review has summarized current mechanisms of intestinal immune tolerance to symbiotic microorganisms from multiple perspectives including Mø cells, DC cells, TLRs, PPAR- , intrinsic microbial characteristics, ALPI, and Treg cells, the intestinal immune system is a complex network, and symbiotic microbial species and colonization processes are complicated. Most existing re-

search involves isolated, single-angle verification. Therefore, the development of systems biology is expected to provide more comprehensive explanations of immune tolerance from a holistic perspective, offering support for in-depth elucidation of microbe-host interactions. Meanwhile, research on intestinal immune tolerance mechanisms to symbiotic microorganisms can provide basic data for scientific feeding of ruminants. Current ruminant nutrition strongly advocates promoting microbial protein synthesis, which may cause changes in intestinal microbiota composition and trigger intestinal immune responses and inflammation. Research on intestinal immune tolerance mechanisms can provide strategies to avoid or suppress such immune reactions. Furthermore, rumen acidosis caused by high-concentrate feeding has become a key issue in ruminant husbandry. High-concentrate feeding affects digestive tract microbiota composition, and research on intestinal immune tolerance mechanisms can provide strategies for selectively improving rumen microbial composition in ruminants, thereby alleviating rumen acidosis without affecting normal digestive metabolism or causing inflammatory diseases.

References

[1] Zhang YM, Tian F, Guo XK. Intestinal lymphoid tissue and symbiotic bacteria jointly maintain intestinal environmental homeostasis[J]. Chinese Journal of Microecology, 2010, 22(9): 854-856.
[2] Xu KJ, Li LJ. Normal intestinal flora and intestinal immunity[J]. Foreign Medical Sciences • Epidemiology and Infectious Disease, 2005, 32(3): 181-183.
[3] Zhu XH. Research progress on immune tolerance mechanisms[J]. Journal of Cellular and Molecular Immunology, 2011, 27(5): 593-595.
[4] Li QY, Zhang SX. Research progress on immune tolerance mechanisms[J]. Immunological Journal, 2002, 18(3): 98-101.
[5] Anderson MS, Su MA. AIRE expands: new roles immune tolerance and beyond[J]. Nature Reviews Immunology, 2016, 16(4): 247-258.
[6] Scandling JD, Busque S, Dejbakhsh-Jones S, et al. Tolerance and withdrawal of immunosuppressive drugs in patients given kidney and hematopoietic transplants[J]. American Journal of Transplantation, 2012, 12(5): 1133-1145.
[7] Correale J, Farez MF. The impact of parasite infections on the course of multiple sclerosis[J]. Journal of Neuroimmunology, 2011, 233(1/2): 6-11.
[8] Arck PC, Hecher K. Fetomaternal immune cross-talk and its consequences for maternal and offspring' s health[J]. Nature Medicine, 2013, 19(5): 548-556.
[9] Wang YX, Yang L, Bi JH, et al. Immunotolerance effect of progesterone in early pregnancy of ruminants[J]. China Animal Husbandry & Veterinary Medicine, 2015, 42(2): 493-497.
[10] Zuo ZY, Zhang C. Research progress on intestinal mucosal immune tolerance mechanisms[J]. Modern Immunology, 2015, 35(1): 68-71.
[11] Castro-Sánchez P, Martin-Villa JM. Gut immune system and oral tolerance[J]. British Journal of Nutrition, 2013, 109(Suppl. 2): S3-S11.
[12] Luo X, Miller SD, Shea LD. Immune tolerance for autoimmune disease and cell transplantation[J]. Annual Review of Biomedical Engineering, 2016, 18:

181-205.
[13] Haziak K, Herman AP, Tomaszewska-Zaremba D. Effects of central injection of anti-LPS antibody and blockade of TLR4 on GnRH/LH secretion during immunological stress in anestrous ewes[J]. Mediators of Inflammation, 2014, 2014: 867170.
[14] Cukrowska B, Kozáková H, Řeháková Z, et al. Specific antibody and immunoglobulin responses after intestinal colonization of germ-free piglets with non-pathogenic Escherichia coli O86[J]. Immunobiology, 2001, 204(4): 425-433.
[15] Zhou F, Zhang GX, Rostami A. Apoptotic cell-treated dendritic cells induce immune tolerance by specifically inhibiting development of CD4+ effector memory T cells[J]. Immunologic Research, 2016, 64(1): 73-81.
[16] Malchow S, Leventhal DS, Lee V, et al. Aire enforces immune tolerance by directing autoreactive T cells into the regulatory T cell lineage[J]. Immunity, 2016, 44(5): 1102-1113.
[17] Wei JC. Fas/FasL-mediated apoptotic cell mechanism and its pathophysiological significance[J]. Foreign Medical Sciences • Surgery, 2003(1): 21-23.
[18] Miyara M, Sakaguchi S. Natural regulatory cells: mechanisms suppression[J]. Trends in Molecular Medicine, 2007, 13(3): 108-116.
[19] Li D, Ren YN, Fan HH. Classification of macrophages and differences in their regulatory functions[J]. Life Sciences, 2011, 23(3): 249-254.
[20] Mantovani A, Sica A, Sozzani S, et al. The chemokine system in diverse forms of macrophage activation and polarization[J]. Trends in Immunology, 2004, 25(12): 677-686.
[21] Chu CY, Wu YH, Zhu L. Research progress on the role and mechanism of macrophages in transplant rejection[J]. Journal of Cellular and Molecular Immunology, 2015, 31(12): 1721-1723, 1727.
[22] Qin HY. Macrophages and immune tolerance[J]. Shanghai Immunology Journal, 1987, 7(6): 369-372.
[23] Smythies LE, Sellers M, Clements RH, et al. Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity[J]. Journal of Clinical Investigation, 2005, 115(1): 66-75.
[24] Sato A, Iwasaki A. Intestinal epithelial barrier and mucosal immunity—Peyer' s patch dendritic cells as regulators of mucosal adaptive immunity[J]. Cellular and Molecular Life Sciences, 2005, 62(12): 1333-1338.
[25] Shu Y. Research progress on intestinal mucosal immune system[J]. Journal of Microbes and Infections, 2007, 2(1): 59-62.
[26] Gu HH. Dendritic cells and immune tolerance[J]. International Journal of Blood Transfusion and Hematology, 2013, 36(4): 464-467.
[27] Dumitriu IE, Dunbar DR, Howie SE, et al. Human dendritic cells produce TGF- 1 under influence of lung carcinoma cells and prime the differentiation of CD4+CD25+Foxp3+ regulatory cells[J]. The Journal Immunology, 2009, 182(5): 2795-2807.
[28] Yang LN, Bian GR, Zhu WY. Interaction between intestinal microbial flora and immune function in monogastric animals[J]. Acta Microbiologica Sinica, 2014, 54(5): 480-486.
[29] Wang S, Charbonnier LM, Rivas MN, et al. MyD88 adaptor-dependent

microbial sensing by regulatory T cells promotes mucosal tolerance enforces commensalism[J]. Immunity, 2015, 43(2): 289-303.
[30] Luo J, Li W, Duan YF, et al. Host discriminates between probiotics and pathogens: impact of receptor 5-flagellin interaction evolution[J]. Microbiology, 2014, 41(7): 1368-1375.
[31] Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis[J]. Nature Reviews Immunology, 2014, 14(3): 141-153.
[32] Lee J, Mo JH, Katakura K, et al. Maintenance of colonic homeostasis by distinctive apical TLR9 signalling in intestinal epithelial cells[J]. Nature Cell Biology, 2006, 8(12): 1327-1336.
[33] Naik S, Kelly EJ, Meijer L, et al. Absence of toll-like receptor 4 explains endotoxin hyporesponsiveness in human intestinal epithelium[J]. Journal of Pediatric Gastroenterology & Nutrition, 2001, 32(4): 449-453.
[34] Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators[J]. Nature, 1990, 347(6294): 645-650.
[35] Fu XY, Huang X. Research progress and application prospects of peroxisome proliferator-activated receptors[J]. China Tropical Medicine, 2008, 8(2): 321-325.
[36] Nencioni A, Wesselborg S, Brossart P. Role of peroxisome proliferator-activated receptor and its ligands in the control of immune responses[J]. Critical Reviews in Immunology, 2003, 23(1/2): 1-13.
[37] von Knethen A, Brüne B. PPARgamma-an important regulator monocyte/macrophage function[J]. Archivum Immunologiae Therapiae Experimentalis, 2003, 51(4): 219-226.
[38] Kelly D, Campbell JI, King TP, et al. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR- and RelA[J]. Nature Immunology, 2004, 5(1): 104-112.
[39] Xu SW, Ying T, Li S. Research progress on PPAR- function[J]. Journal of Northeast Agricultural University, 2011, 42(9): 1-6.
[40] Wang AL, Wu QB, Sun QL. Interaction mechanism between intestinal flora and intestinal mucosal immune system[J]. Chinese Journal of Microecology, 2009, 21(4): 382-385.
[41] Golenbock DT, Hampton RY, Qureshi N, et al. Lipid A-like molecules that antagonize the effects of endotoxins on human monocytes[J]. The Journal of Biological Chemistry, 1991, 266(29): 19490-19498.
[42] Kim EE, Wyckoff HW. Reaction mechanism of alkaline phosphatase based on crystal structures: two-metal ion catalysis[J]. Journal of Molecular Biology, 1991, 218(2): 449-464.
[43] Alam SN, Yammine H, Moaven O, et al. Intestinal alkaline phosphatase prevents antibiotic-induced susceptibility enteric pathogens[J]. Annals Surgery, 2014, 259(4): 715-722.
[44] Qureshi OS, Zheng Y, Nakamura K, et al. Trans-endocytosis of CD80 and CD86: a molecular basis extrinsic function CTLA-4[J]. Science, 2011, 332(6029): 600-603.

[45] Liang BT, Workman C, Lee J, et al. Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class [J]. The Journal of Immunology, 2008, 180(9): 5916-5926.
[46] Mandapathil M, Lang SE, Gorelik E, et al. Isolation of functional human regulatory T cells (Treg) from the peripheral blood based on the CD39 expression[J]. Journal of Immunological Methods, 2009, 346(1/2): 55-63.
[47] Shin JY, Yoon IH, Lim JH, et al. CD4+VEGFR1high T cell as a novel Treg subset regulates inflammatory bowel disease lymphopenic mice[J]. Cellular & Molecular Immunology, 2015, 12(5): 592-603.
[48] Bae MJ, Shin HS, See HJ, et al. Baicalein induces CD4+Foxp3+ T cells and enhances intestinal barrier function a mouse model of allergy[J]. Scientific Reports, 2016, 6: 32225.

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