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Review Article

Gut health: The results of microbial and mucosal immune interactions in pigs

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ABSTRACT

There are a large number of microorganisms in the porcine intestinal tract. These microorganisms and their metabolites contribute to intestinal mucosal immunity, which is of great importance to the health of the host. The host immune system can regulate the distribution and composition of intestinal microorganisms and regulate the homeostasis of intestinal flora by secreting a variety of immune effector factors, such as mucin, secretory immunoglobulin A (sIgA), regenerating islet-derived III (RegIII)γ, and defensin. Conversely, intestinal microorganisms can also promote the differentiation of immune cells including regulatory T cells (T_{reg}) and Th17 cells through their specific components or metabolites. Studies have shown that imbalances in the intestinal flora can lead to bacterial translocation and compromised intestinal barrier function, affecting the health of the body. This review focuses on the composition of the pig intestinal flora and the characteristics of intestinal mucosal immunity, discusses the interaction mechanism between the flora and intestinal mucosal immunity, as well as the regulation through fecal microbiota transplantation (FMT), dietary nutritional composition, probiotics and prebiotics of pig intestinal microecology. Finally, this review provides insights into the relationship between intestinal microorganisms and the mucosal immune system.

1. Introduction

Modern pig production faces many persistent challenges, such as weaning diarrhea due to changes in diet and living environment (Markowiak and Slizewska, 2018), and the abuse of growth-promoting antibiotics in feed (Van Boeckel et al., 2019). In the past decade, through advancements in 16S rRNA sequencing and metagenomics analyses in addition to other microbiology technologies, it has become apparent that the homeostasis of intestinal flora plays an important role in host health (Isaacson and Kim, 2012; Nowland et al., 2019; Yin et al., 2020). Intestinal microorganisms metabolize dietary nutrients ingested by the host and ferment complex plant polysaccharides that cannot be directly utilized by the host, thus providing energy for the host. In addition, the microbiota has been shown to synthesize vitamin K, regulate choline metabolism, and stimulate the development and maturation of the intestinal immune system (Tremaroli and Backhed, 2012). Conversely, imbalances in the intestinal microflora have been associated with obesity, fatty liver, diabetes, inflammatory bowel disease, constipation, and other diseases (Portune et al., 2017; Sommer and Backhed, 2013; Yin et al., 2018).

There are a large number of immune tissues and cells in the intestinal tissue of pigs, including lymph nodes, Paneth cells, and innate lymphocytes (Kim and Ho, 2010; Pabst et al., 2016). Under normal physiological conditions, the intestinal immune system plays a vital role in the homeostasis of intestinal microorganisms (Thaiss et al., 2016). In piglets, incomplete development of intestinal organs, low autoimmunity, and drastic changes in food sources as a result of weaning quickly alter intestinal homeostasis, resulting

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in diarrhea. In finishing pigs with a more mature intestinal immune system, dietary changes do not have an obvious effect on intestinal dysfunction (Frese et al., 2015). During the period of intestinal flora colonization of piglets, transplantation of exogenous bacteria is able to increase the abundance of beneficial intestinal bacteria, significantly improving the intestinal barrier function of piglets, and ultimately alleviating the occurrence of weaning diarrhea (Cheng et al., 2018; Hu et al., 2018b).

An increasing number of studies on intestinal microecology and microflora have reported links between the immunity of intestinal microflora and intestinal mucosa health, as well as the entire body (JF and MT, 2020; Round and Mazmanian, 2009). Intestinal microorganisms can regulate the host intestinal epithelial barrier and immune barrier through flagellin, lipopolysaccharide (LPS) and metabolites, promote the development and maturity of the host immune system, and improve intestinal barrier function (Hooper et al., 2012; Powell et al., 2017; Sonnenberg and Artis, 2019). Here, the composition of intestinal microflora, as well as the composition and responses of the intestinal mucosal immune system in pigs are introduced, and the possible mechanism of the interaction between microflora and intestinal mucosal immunity will be discussed.

2. Composition of intestinal microorganisms in pigs

There are many types of microorganisms in the digestive tract of mammals, including bacteria, fungi, archaea, protozoa, and a number of viruses, with a total of at least 10^{14} (Clemente et al., 2012; Sommer and Backhed, 2013). Intestinal microorganisms are predominantly bacteria, consisting of thousands of species. Some of the most prominent genera include Firmicutes and Bacteriodetes, followed by Proteobacteria, Verrumicrobia, Actinobacteria (Donaldson et al., 2016; Human Microbiome Project, 2012). Newborn piglets gradually establish intestinal flora due to contact with sow vaginal and fecal microorganisms. These exposures come in the form of direct contact with the birth canal of sows during delivery, and sow feces during normal behaviors (Kim and Isaacson, 2015). During lactation, sow's milk provides nutritional advantages for the colonization of *Lactobacillus,* and establishes a milk-oriented microbiome (Frese et al., 2015). From 7 through to 21 d of age (birth to early weaning), more than 95% of the microorganisms in the intestines are strictly anaerobic (Firmicutes, Bacteriodetes, and Proteobacteria) (Slifierz et al., 2015). The abundance of Firmicutes (54.0%), Bacteroidetes (38.7%) and Proteobacteria (4.2%) at the phyla level changes to Bacteroidetes (59.6%), Firmicutes (35.8%) and Proteobacteria (1%) after weaning. This indicates that the phyla of microorganisms were essentially the same pre- and post-weaning, although the relative abundance changed significantly, where the proportion of Bacteroidetes increased (Alain et al., 2014).

The composition of organisms is affected by both dietary composition and weaning stress. It was reported that, especially after rapid weaning, the microbial diversity and the number of *Lactobacillus* bacteria decreased, while the abundance of *Clostridium* spp., *Prevotella* spp., and *Escherichia coli*, increased (Gresse et al., 2017). The abundance of bacteria in *Bacteroides* was the highest pre-weaning, whereas the highest levels of *Prevotella* and *Clostridium* were reported post-weaning. During the weaning transition period, a significant shift from *Bacteroides* to *Prevotella* was reported (Alain et al., 2014). It is possible that *Bacteroides* aid in the digestion of monosaccharides and oligosaccharides in breast milk pre-weaning, whereas *Prevotella* enhance the degradation of plant polysaccharides in feed, such as hemicellulose and xylan (Alain et al., 2014; Lamendella et al., 2011). Therefore, when the dietary composition of pigs is switched to plant carbohydrates, *Prevotella*-related microorganisms in the intestines proliferate (Slifierz et al., 2015).

Similar to other mammals, the intestinal microbial richness of pigs also varied significantly with the intestinal segment. The ileum is mainly colonized by Firmicutes and Proteobacteria, and the abundance of Bacteroidetes in the cecum and colon is significantly increased (Looft et al., 2014; Zhang et al., 2018). Furthermore, the microflora of the intestinal lumen is very different from that of the intestinal mucosa. The richness of *Bacteroides* in the lumen of the colon (40.09%) is much higher than that of the small intestine (1.69%). The genera found in the intestinal lumen primarily consist of Prevotellaceae, Ruminococcaceae, Lachnospiraceae and Veillonellaceae. Prevotellaceae, Enterobacteriaceae, Caulobacteraceae, Enterococcaceae, Xanthomonadaceae and Pseudomonadaceae dominate the intestinal mucosa (Zhang et al., 2018).

The composition of intestinal microorganisms in pigs is affected by diet, antibiotics, body weight, physiological status, genetics, and the living environment (Isaacson and Kim, 2012). Among these factors, diet is the most important. Corn fiber (neutral detergent fiber) contains plant lignin, hemicellulose, cellulose, and other structural components that pigs alone cannot digest. These nutrients enter the intestine and are fermented into bioavailable molecules by the colonic microbiota (Wang et al., 2019f). The intestinal flora regionally colonizes the gut in specific spaces suitable for their survival. The resulting interactions with the host form a dynamic yet balanced complex ecosystem.

3. Response mechanism of intestinal mucosal immune system

The mucosal immune system exists in various anatomical locations of the body, such as the respiratory, reproductive, or digestive tracts, and plays a complex role in defense of the host (Iijima et al., 2001). In the intestinal tract, the mucosal immune system plays an important role. Not only does it contribute to digestion and absorption of nutrients, it also serves as an important barrier for the body to resist pathogenic insult (Knoop and Newberry, 2018). The intestinal mucosal immune response is induced by the local mucosal tissues, where immunoreactive cells are stimulated by pathogens and other antigens. Mucosal immunity is characteristically defined by the production of secretory immunoglobulin A (sIgA) by plasma cells (Iijima et al., 2001). Intestinal epithelial cells (IEC) are the first line of defense against antigens within the intestinal lumen, and are composed of a variety of cell types (enterocytes, goblet cells, neuroendocrine cells, cluster cells, Paneth cells and Microfold cells). These different epithelial cell types cooperate to maintain intestinal homeostasis and promote host defenses (Allaire et al., 2018).

The intestinal mucosal immune system can be divided into induction and effector sites according to tissue structure and function. The induction site mainly refers to gut-associated lymphoid tissue (GALT), which is composed of highly organized lymphoid follicles known as Peyer's patches (PP), mesenteric lymph nodes (MLN), and isolated lymphoid follicles (ILF). The immune cells in the induction sites include Microfold cells and antigen presenting cells (APC), which uptake and present antigens to immune effector cells (Pabst and Rothkotter, 2006). Some types of APC include dendritic cells (DC), macrophages and IEC (Allaire et al., 2018). Effector sites are the diffuse distribution of lymphocytes in the epithelial layer and lamina propria, including intestinal mucosal intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL). The presentation of antigens by APC to these cells results in the production of antibodies and various immunomodulatory cytokine factors (Allaire et al., 2018; Castro and Arntzen, 1993; Nagura and Sumi, 1988; Pabst and Rothkotter, 2006).

The intestinal mucosal immune response is induced by the extraction of antigens from the induced site. The main induction site of the intestinal mucosal immune system is the PP, in which the antigen transporting cells (Microfold cells), make up the surface of the PP (Allaire et al., 2018). Histologically, Microfold cells are characterized by short microvilli, small cytoplasmic vesicles and a few lysosomes. These cells are capable of phagocytosing microorganisms and complex antigens, and can ingest and transport intraluminal antigens such as proteins and other small particles (viruses, parasites and microspheres) to APC in the subepithelial lymphoid tissue (Kobayashi et al., 2019; Neutra et al., 1996; Yamamoto et al., 2012). Next, the antigens are presented to antigen receptor molecules, and are finally presented to helper T lymphocytes. Helper T lymphocytes secrete a variety of lymphokines to induce B lymphocytes, which then differentiate and proliferate to produce a large amount of sIgA, which is released into the intestinal lumen, affecting its immunological properties (Iijima et al., 2001; Yamamoto et al., 2012).

In the wall of the porcine small intestines, lymphoid tissue exists in the form of isolated lymphoid follicles, or as high tissue lymphoid follicles (such as PP) (Stokes, 2017). Under the regulation of the immune system, Microfold cells in the jejunum and ileum take up intestinal lumenal antigens (Rothkotter, 2009). In the porcine colon, the immune tissue is known as the lymphoglandular complex (Morfitt and Pohlenz, 1989). Compared with germ-free piglets, the proportion of B cells in the ileum of conventional piglets was higher, IgA^+ B cells were more common, distributed evenly in the whole small intestine, and the ratio of effector to memory $CD4^+CD8^+$ αβ Th cells was significantly higher than that of germ-free piglets (Potockova et al., 2015). At birth, the mucosal immune system of piglets is underdeveloped, and the number of white blood cells in the lamina propria of the intestine is very small. Sow's milk is critical to the promotion and development of the neonatal immune system (Salmon et al., 2009). As the pigs age, the immune system will develop, in a manner that is driven by environment, diet, genotype, and intestinal microbes (Stokes, 2017).

4. Mucosal immune system regulates intestinal microbiota

4.1. Stratification and distribution

The intestinal immune system can limit direct contact between bacteria and the intestinal cell epithelium, thus reducing the possibility of pathological interactions. This occurs through 2 mechanisms. First, the intestinal barrier minimizes direct contact between intestinal microorganisms and the surface of epithelial cells. Second, the immune system limits bacteria to specific intestinal sites, reducing their exposure to the systemic immune system (Hooper et al., 2012; Yin et al., 2019). The regulation of the intestinal immune system on the stratification and distribution of microorganisms is accomplished via the combined action of epithelial cells, mucus, and antimicrobial peptides (Fig. 1) (Hooper, 2015; JF and MT, 2020).

The goblet cells of the intestinal epithelium secrete mucus, which forms a layer of elastic gel that covers the epithelial surface, isolating the intestinal epithelium from the environment of the intestinal lumen. This greatly reduces the contact between the microbiota and the host tissue, protecting the parenchyma from luminal microorganisms and antigens, and preventing microbial translocation (Knoop and Newberry, 2018; Propheter et al., 2017). The layering of mucus over the small intestine is not obvious; there is only a thin and intimate mucus layer. In the colon, there are 2 structurally distinct mucus layers; the outer is loose, and the inner is adherent and tightly compact. The outer layer is where the symbiotic intestinal microflora colonize and interact with oligosaccharides on the mucin. The inner mucus layer is resistant to bacterial penetration, and has almost no bacterial colonization (Donaldson et al., 2016; Johansson et al., 2014; Kim and Ho, 2010). The outer mucus layer is colonized with *B. acidifaciens*, *Bacteroides fragilis*, Bifidobacteriaceae and *Akkermansia muciniphila*, inner mucus layer includes *B. fragilis* and *Acinetobacter* spp. (Donaldson et al., 2016).

The mucus secreted by intestinal goblet cells is mainly composed of mucin 2 (MUC2), intestinal trefoil factor 3 (TFF3), resistin-like molecule β (RELM β) and Fc-γ binding protein (Fc-γ BP) (Kim and Ho, 2010; Vaishnava et al., 2011). The MUC2 protein is the most abundant mucin in the intestinal tract. Goblet cells secrete mucin under the stimulation of many substances (including acetylcholine, histamine, and prostaglandin). The mucin is released to the external mucus layer, which competitively binds to the adhesin receptors of epithelial cells and inhibits the adhesion and colonization of pathogenic microorganisms in the intestinal tract (Knoop and Newberry, 2018). MUC2, Fc- γ BP, and TFF3 proteins then combine through covalent bonds to promote the secretion of soluble mucus, increase mucus viscosity, and function synergistically to protect and maintain the integrity of the intestinal mucosa (Yu et al., 2011). When the secretion of MUC2 and TFF3 decreases, harmful microorganisms can easily penetrate the mucus layer and colonize the intestinal epithelium, which results in epithelial cell damage (Lidell et al., 2006). RELM β is an antibacterial protein that limits the contact between Gram-negative bacteria and the surface of the colonic epithelium. In addition, RELM β can bind negatively charged lipids on the surface of bacterial cell membranes to form polymer pores to lyse bacterial cells. In mice lacking RELM β, the abundance of Proteus increased in the internal colonic mucus layer (Propheter et al., 2017).

In addition to goblet cells, other IEC secrete antibacterial proteins, among which Regenerating islet-derived III (RegIIIγ) and sIgA are essential in the isolation of microorganisms in the intestinal mucosa (Vaishnava et al., 2011). A C-type lectin, RegIIIγ is expressed by IEC, which forms a sterile isolation zone approximately 50 μm out from the intestinal epithelial tissue to restrict the contact between Gram-positive bacteria and the intestinal surface. In the intestine of RegIIIγ knockout mice, it was reported that the separation between intestinal mucosa and bacteria disappeared, which resulted in increased bacterial colonization of the intestinal epithelial surface (Vaishnava et al., 2011). The antibacterial mechanism of RegIII γ is mainly through binding to peptidoglycan on the surface of the Gram-positive bacterial cell wall to destroy the cell wall and membrane, resulting in cytoplasmic leakage and bacterial lysis (Cash et al., 2006). Plasma cells in the intestinal lamina propria produce sIgA, and are the main effector in the mucosal response. These immunoglobulins serve to neutralize bacterial toxins in the intestinal cavity and protect the mucosal surface, thus resisting pathogen infection and maintaining immune stability (JW et al., 1996; NJ et al., 2011; Pabst et al., 2016).

4.2. Composition of intestinal microorganisms

In recent years, it has been reported that antimicrobial peptides in the intestinal mucosal immune system play an important role in the regulation of the intestinal flora structure (Ganz, 1999; Ulm et al., 2012). At present, a variety of antimicrobial peptides have been identified in porcine intestines, including defensins, cathelicidins, hepcidin (liver expressed antimicrobial peptide-2), peptidoglycan recognition proteins (PGRP), liver expressed antimicrobial peptide-2 (LEAP-2), and NK-lysins (Sang and Blecha, 2009; Sang et al., 2006). The majority of recent studies have focused on the antibacterial mechanisms of defensin and cathelicidin proteins.

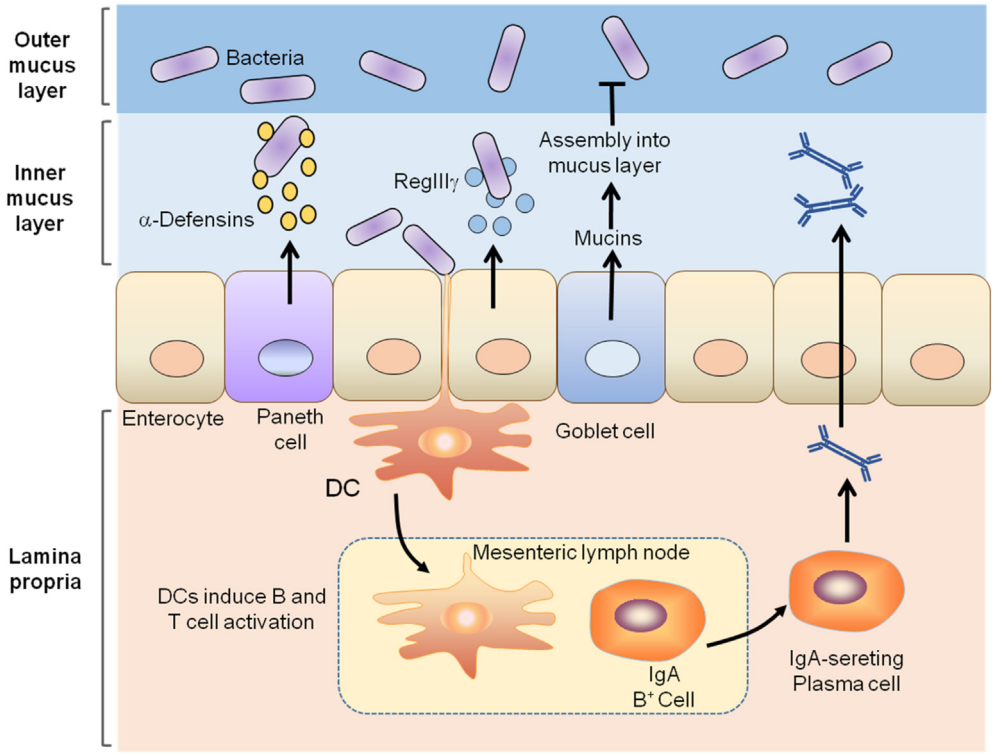

Fig. 1. Regulation of intestinal microorganisms by the mucosal immune system. The intestinal immune system regulates the stratification and distribution of intestinal microorganisms through the combined action of epithelial cells, mucus, and antimicrobial peptides. Goblet cells secrete mucus, limiting direct contact between microbes and the intestinal epithelium and preventing microbial translocation. Intestinal cells and goblet cells can secrete antibacterial proteins such as α-defensins and regenerating islet-derived III (RegIII)γ. Dendritic cells (DC) in the intestinal lamina propria can engulf some bacterial antigens that penetrate the mucous layer and present them in the mesenteric lymph nodes. DC induce B lymphocytes to differentiate into plasma cells that secrete large quantities of immunoglobulin A (IgA) into the intestinal cavity.

There are 3 classes of mammalian defensins, including α-, β-, and θ-defensins (Sang and Blecha, 2009). Secreted by neutrophils and IEC, defensins exhibit broad-spectrum antibacterial activity against Gram-positive and sexual bacteria, and in some cases, against fungi, viruses and protozoa (Selsted and Ouellette, 2005). At the time of publication, only porcine β-defensins (pBD) have been identified in pigs (Myer, 1982). The pBD contains 6 cysteine residues and can form 3 pairs of disulfide bonds, with pBD-1 and pBD-2 being the most studied (Yeaman and Yount, 2007). In vitro, pBD-1 showed broad-spectrum antibacterial activity when prepared in a 10 mM sodium phosphate buffer (pH7.4), and effectively killed pathogenic bacteria such as *E. coli*, *Salmonella typhimurium*, *Listeria monocytogenes* and *Candida albicans* (Shi et al., 1999). The treatment of *S. typhimurium* with a low dose of pBD-2 resulted in retraction of the bacterial cell membrane. When the concentration of pBD-2 is close to the minimum bactericidal concentration (MBC), it resulted in leakage of the bacterial cytoplasm and the complete dissolution of cells (EJ et al., 2008).

The family cathelicidin protein was the first class of mammalian antimicrobial peptides to be isolated (Sang and Blecha, 2009; Zhang et al., 2000). At present, 11 types of antimicrobial porcine cathelicidin peptides have been characterized, including amino acid peptide (PR-39) which is rich in proline and arginine, prophenin-1 (PF-1) that is rich in proline and phenylalanine, PG1 to PG-5, PF2 (rich in cysteine), and 3 porcine bone marrow antimicrobial peptides PMAP-23, PMAP-36, and PMAP-37 (Zanetti, 2005). Among these, PR-39 exhibits potent antibacterial effects on pathogenic gut bacteria, such as *Enterococcus faecalis*, *E. coli* and *Bacillus subtilis* (Veldhuizen et al., 2014). Lethal effect of PR-39 may be achieved by preventing protein synthesis and inducing the degradation of some proteins needed for DNA replication (Boman et al., 1993). PMAP-23 is a porcine antimicrobial peptide discovered in 1994 (Zanetti et al., 1994), which is rich in arginine and contains 2 α-helical regions. The C-terminal is lipophilic, can be inserted into cells to break cells, and has anti-Gram-positive and Gram-negative bacteria (Liu et al., 2020).

Although the above-described peptides exert antibacterial activity, the immunomodulatory and barrier protective effects of the antimicrobial peptides have not been thoroughly examined. At present, in order to reduce the use of antibiotics, antimicrobial peptides are intriguing and potential veterinary drugs for the prevention and treatment of diarrhea in piglets. The extraction and purification of natural antimicrobial peptides is very difficult, but can be obtained by artificial design, chemical synthesis or genetic engineering. It is also necessary to solve industrial bottlenecks such as poor stability in the gastrointestinal tract, low efficiency of oral absorption, and processing technology.

5. Regulation of intestinal microorganisms by the mucosal immune system

5.1. Intestinal lymphoid tissue development and epithelial barrier

In recent years, it has been demonstrated that intestinal flora is critical in the regulation of both the innate and adaptive immune systems (Honda and Littman, 2016; Thaiss et al., 2016). The germ-free mouse model is often used to explain how microflora in the digestive tract affects the development of the immune system. In the germ-free animal model, the intestinal immune system is immature, the intestinal lamina propria lacks plasma cells, and the number of T cells is strikingly low (Cebra, 1999). In addition, lymphoid follicles isolated from the small intestine of germ-free

mice were not developed, and sIgA as well as CD8 α β IEL were lacking (Hooper et al., 2012). Compared with conventionally raised pigs, germ-free pigs have abnormal intestinal morphology, longer villi, lower crypt depth, lower crypt cell proliferation, and lower intestinal cell turnover (Willing and Van Kessel, 2007). In the preterm germ-free piglets, the mucus layer was thinner and the stratification was not obvious, the density of Lamina propria and submucosa cells of the ileum decreased, the fibrous tissue was loose and sparse, containing immature mesenchymal components, and the PP were smaller (Splichalova et al., 2018).

Microorganisms also play an important role in the regulation and repair of the physical intestinal barriers. For example, the probiotic *E. coli* strain Nissle 1917 enhances epithelial barrier function by up-regulating intestinal tight junction protein zonula occludens 1 (ZO-1), thus reducing intestinal epithelial permeability, and improves the incidence of gastrointestinal diseases (Ukena et al., 2007). Piglets fed *Lactobacillus frumenti* during d 6 to 20 before early weaning can significantly upregulate the expression of intestinal tight junction proteins (*ZO-1*, occludin and claudin-1), and had a significant increase in the levels of intestinal sIgA (Hu et al., 2018a).

Intestinal microbial pathogen associated molecular patterns (PAMP) such as LPS or flagellin can promote the secretion of the antibacterial protein RegIIIγ by IEC by activating the toll-like receptors-myeloid differentiation primary response 88 (TLR-MyD88) signaling pathway, which also appears to promote the repair of damaged IEC (Hooper et al., 2012; Vaishnava et al., 2011; Zhu et al., 2018). In addition, bacterial flagellin stimulates the production of interleukin (IL)-23 through the activation of TLR5, which is expressed on $CD103^{+}CD11b^{+}$ DC in the lamina propria. This in turn promotes the expression of *IL-22* in innate lymphoid cells, which further stimulates the production of RegIIIγ (Kinnebrew et al., 2012; Macpherson and Ganal-Vonarburg, 2018). These examples illustrate the importance of microorganisms in inducing the development of the host immune system, but may represent only a small part of the many effects of the microflora on the host immune system. Moreover, current research has been limited to a small number of microorganisms, and the role of a large number of microbial populations in the intestinal barrier requires further exploration.

5.2. Regulation of T cell differentiation

A large number of immune cells reside within the lamina propria of the intestinal tract. These cells include Th1 cells that produce interferon (IFN), Th17 cells that produce IL-17a, IL-17f and IL-22, innate lymphoid cells that produce various cytokines and $Foxp3^{+}$ regulatory T cells (T_{reg}) (Hooper et al., 2012). Under the action of intestinal microorganisms, pro-inflammatory and anti-inflammatory cells balance the responses, and jointly maintain the homeostasis of intestinal mucosa (Fig. 2).

A subgroup of effector helper T cells, Th17 cells, secrete cytokines such as IL-17, IL-22 and IFN-γ (Bellone et al., 2020). However, Th17 cells have been reported to contribute immunopathologic effects in many inflammatory diseases, but also serve to maintain the integrity of the intestinal barrier in a non-inflammatory manner (Lecuyer et al., 2014; Omenetti et al., 2019). The differentiation of Th17 cells in the lamina propria of the small intestine requires the participation of specific bacteria (Ivanov et al., 2008). Induction of Th17 cells by *Citrobacter rodentium* are inflammatory and mainly participate in aerobic glycolysis, which is usually related to inflammatory effector cells (Omenetti et al., 2019). The Th17 cells induced by segmented filamentous bacteria (SFB) are non-inflammatory and play an important role in the maintenance of intestinal homeostasis, the development of mucosal structure, the induction of IgA, and the maturation of postpartum intestinal immune function (Schnupf et al., 2013, 2017). Further, SFB can induce the expression of serum amyloid A (*SAA*) in the small intestine, and SAA also promotes the secretion of transforming growth factor (TGF)-β and IL-6 by DC. The synergistic effect of TGF-β and IL-6 is the key factor to initiating differentiation of Th17 cells (Ivanov et al., 2008). It has also been reported that SFB can transfer antigens to IEC and regulate host Th17 cell homeostasis through microbial adhesion-triggered endocytosis (Ladinsky et al., 2019).

Analysis by electron tomography showed that SFB could directly adhere to IEC, triggering the formation of endophagocytic vesicles at the adhesion site. These endocytic vesicles contained SFB cell wall derived bacterial proteins that induce the activation of Th17 cells. The endocytosed SFB proteins are then shuttled through the IEC endosomal lysosomal network, and induce the activation of lamina propria antigen-specific Th17 cells (Atarashi et al., 2015; Kumar et al., 2016; Ladinsky et al., 2019). This study suggests a new way for IEC to obtain antigens from intestinal symbionts and how microbial proteins communicate with the host to maintain mucosal T cell homeostasis. Although there are few Th17 cells in the lamina propria of germ-free mice (Ivanov et al., 2008), those inoculated with SFB exhibit increased differentiation and accumulation of Th17 cells, which results in increased numbers of Th1 cells (Gaboriau-Routhiau et al., 2009; Ivanov et al., 2009). Furthermore, SFB induces intestinal innate lymphoid cell 3 (ILC3) to produce IL-22, induces epithelial expression of *SAA*, and promotes Th17 cell differentiation and expression of *IL-17* (Sano et al., 2016).

Regulatory T cells play an important role in adaptive immune tolerance (Russler-Germain et al., 2017). $FOXP3^{+}$ regulatory T cells ($FOXP3^{+}$ T_{reg}) are a subtype of T cells that express CD4, CD25, and the transcription factor FOXP3. Although T_{reg} cells expressing CD4 and Foxp3 are found in many organs throughout the body, the proportion is relatively high in the intestinal and colonic lamina propria (Honda and Littman, 2016), and is likely the key to regulating intestinal inflammation (Smith et al., 2013). Microorganisms can enhance the anti-inflammatory effect of the adaptive immune system by promoting the differentiation of T_{reg} or through the induction of *IL-10* expression (Russler-Germain et al., 2017). *B. fragilis* and clostridial species can induce T_{reg} (Atarashi et al., 2011; JL and SK, 2010). *B. fragilis* exerts a beneficial immunomodulatory effect on the host immune system through capsular polysaccharide A (Deniz and Kasper, 2018), which acts on the heterodimer of TLR2/TLR1 on the surface of antigen-presenting cells, and the C-type lectin-like receptor Dectin-1. Binding to these receptors activates the PI3K signaling pathway, which results in the induction of host T_{reg} to produce immunomodulatory cytokine IL-10 (Erturk-Hasdemir et al., 2019). *Clostridium* can induce colon T_{reg} and play a central role in inhibiting inflammatory and hypersensitivity reactions (Atarashi et al., 2011). Colonization of gnotobiotic mice with a mixture of IV, XIVa and XVIII *Clostridia* strains isolated from mouse feces resulted in an increase in the number of T_{reg} in the colonic lamina propria (Atarashi et al., 2013). At the same time, *Clostridium* strains were also demonstrated to promote the expression of *IL-10* and cytotoxic T lymphocyte protein 4 in T_{reg} cells. As a result, mice with high abundance of *C. enterocolitica* were resistant to colitis (Atarashi et al., 2011, 2013).

Metabolism by microbes in the intestinal lumen can produce butyrate, which has been demonstrated to promote the differentiation of T_{reg} (Furusawa et al., 2013; N et al., 2013; Smith et al., 2013). Butyrate is produced by bacterial fermentation of dietary fiber, which is condensed from 2 acetyl-CoA molecules to acetoacetyl-CoA, and further reduced to butyryl-CoA. This molecule is then converted to butyrate via the phosphate transbutyrylase/butyrate kinase pathway (A et al., 2016; Liu et al., 2018). Butyrate can directly act on the immune cells in the intestinal mucosa,

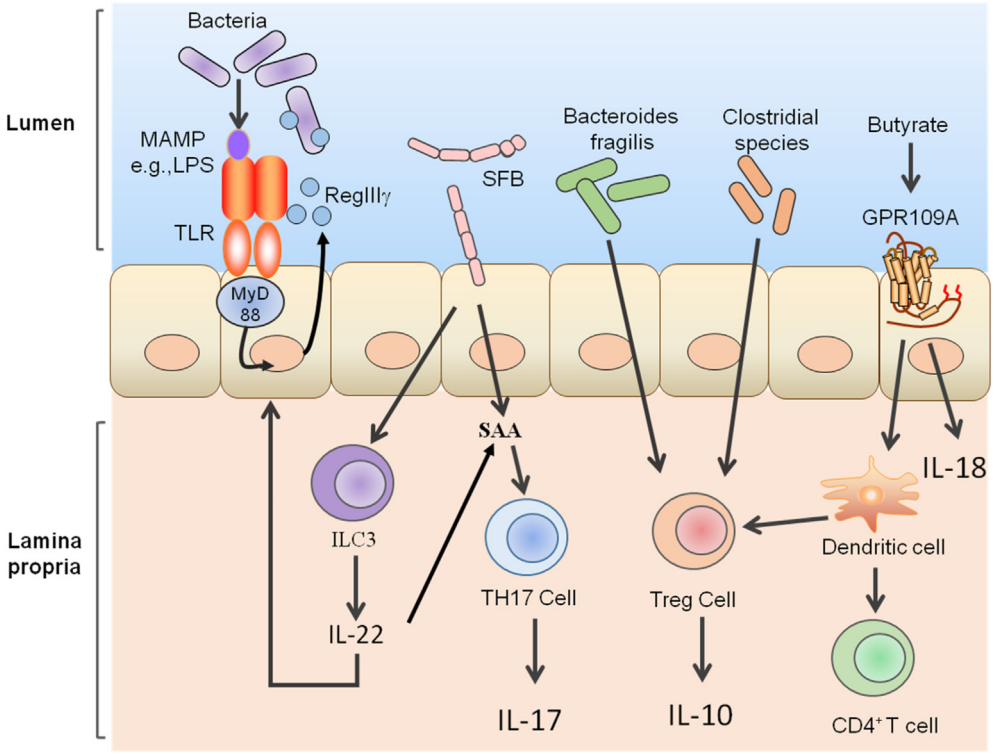

Fig. 2. Effect of intestinal microorganisms on the mucosal immune system. MAMP = microbe-associated molecular pattern; LPS = lipopolysaccharide. Intestinal microbial signals such as flagellin can promote the secretion of the antibacterial protein regenerating islet-derived III (RegIII)γ by intestinal epithelial cells through activation of the toll-like receptors myeloid differentiation primary response 88 (TLR-MyD88) signaling pathway. This promotes the repair of damaged intestinal epithelial cells. Segmented filamentous bacteria (SFB) can induce the expression of serum amyloid A (SAA) in the small intestine, thus promoting the differentiation of Th17 cells. In addition, SFB can induce intestinal innate lymphoid cell 3 (ILC3) to produce interleukin (IL)-22, induce epithelial expression of SAA, and promote the expression of IL-17 in Th17 cells. *B. fragilis* and *Clostridial spp.* Can induce regulatory T cells (T_{reg}) differentiation. Butyrate, the product of bacteria fermentation of dietary fiber, induces the expression of IL-18 in intestinal epithelial cells (IEC) through G protein-coupled receptor 109 A (GPR109A) signaling. In addition, butyric acid can also promote the anti-inflammatory properties of colon dendritic cells through GPR109A signaling, enabling them to induce the differentiation of T_{reg} cells and IL-10-producing $CD4^+$ T cell cells.

increase the number and activity of T_{reg}, and inhibit the activity of neutrophils, macrophages, DC, and effector T cells (Gonçalves et al., 2018). In addition, it has been reported that butyrate promotes the anti-inflammatory properties of colon DC through G protein-coupled receptor 109 A (GPR109A) signaling, which induce the differentiation of T_{reg} cells and $CD4^+$ T cells. Butyrate also directly induces the expression of *IL-18* in IEC through GPR109A signaling (Singh et al., 2014). Metabolomic analyses showed that the concentration of butyrate and the number of T_{reg} cells in the colon were positively correlated. Similarly, it was demonstrated that butyrate induced T_{reg} differentiation both in vitro and in vivo, and alleviated colitis in mice (Furusawa et al., 2013).

5.3. Regulation of flora metabolism nutrients on mucosal immunity

Dietary protein releases a large amount of tryptophan during digestion, and the intestinal flora converts it into indole and its derivatives (Roager and R, 2018; Zelante et al., 2013). Many of these indole derivatives are ligands of an aryl hydrocarbon receptor (AhR), such as indolealdehyde (IAld), indoleacetic acid (IAA), indolepropionic acid (IPA) and indole-3-acetaldehyde (IAAld) (Alexeev et al., 2018; Allison et al., 2018). Signaling by AhR is considered to be a key component of the barrier immune response, and is essential for intestinal epithelial renewal, maintenance of barrier integrity, and intestinal homeostasis (Lamas et al., 2018).

Lactobacillus spp. In the intestinal tract (such as *Lactobacillus reuteri*) can sense dietary tryptophan, rapidly promote its own proliferation, and produce the AhR ligand IAld, which activates the AhR-IL-22 axis. This pathway has been demonstrated to promote the colonization of a diverse flora, inhibiting colonization by *C. albicans*, and protecting mucous membrane from inflammation (Zelante et al., 2013). This study shows that the metabolites produced by intestinal flora metabolizing tryptophan are very important for balancing the intestinal immune system in mammals, and that intestinal flora can affect the symbiosis of host microorganisms through dietary tryptophan. In a recent study on pig production, fecal bacterial suspensions from healthy adult pigs were injected into newborn piglets, and it was found that it could regulate the diversity and composition of colonic flora in the piglets. It was also reported that the indole derivatives (IAA and IAld) produced by the colonic flora increased significantly. This in turn upregulation *IL-22* expression and enhanced the activation of AhR in the piglet colonic mucosa to improve intestinal barrier function (S et al., 2018).

Butyrate also serves as the main energy source of IEC (A et al., 2016; T et al., 2012). Butyrate can inhibit the activity of histone deacetylase 3 (HDAC3), thus inducing metabolic changes of macrophages to increase the expression of antimicrobial peptides and enhance their bactericidal effect (J et al., 2019). In addition, urolithin A (UroA), produced by berry and pomegranate polyphenols is metabolized by intestinal flora, and can also activate AhR. It has been found that oral administration of UroA reduces systemic inflammation, increases epithelial tight junction protein expression, and prevents and relieves colitis in mice (Singh et al., 2019).

Table 1
Effects of microflora intervention on intestinal mucosal immunity in pigs.

Age of pig	Breeds of pig	Microbial species	Mode of intervention	Design	Conclusions	Reference
6 to 20 d	Landrace × Yorkshire crossbred piglets	*Lactobacillus gasseri* LA39	Oral administration	Control group - oral PBS, LA39 - oral administrated of bacterial suspension (*L. gasseri* LA39 in PBS)	LA39 activates the oxidative phosphorylation pathway and increases the energy production in porcine intestinal epithelial cells	Hu et al. (2018c)
6 to 20 d	Landrace × Yorkshire crossbred piglets	*Lactobacillus frumenti*	Oral gavage	Control group - sterile PBS, *L. frumenti* group - PBS suspension containing *L. frumenti* (10^8 CFU/mL)	The levels of intestinal sIgA, intestinal tight junction protein (including ZO-1, occludin and claudin-1) and interferon γ were significantly increased in *L. frumenti* group	Hu et al. (2018a)
1 to 14 d	Duroc × Landrace × Yorkshire crossbred piglets	Chinese adult Jinhua pigs faecal microbiota suspension	FMT	FMT group - oral FMT, control group - orally inoculated with sterile PBS	FMT promotes IAA production in colonic lumen, enhance the activation of AhR and up-regulates *IL-22* expression	Geng et al. (2018)
Days 70 and 100 of gestation	Large White × Landrace pregnant sows	Highly feed-efficient pigs faecal microbiota suspension	Gastric intubation	FMT group - FMT on d 70 and 100 of gestation	FMT group offspring have more goblet cells in the intestinal villi	McCormack et al. (2019)
1 to 28 d	Large White × Landrace piglets	*L. plantarum* ZLP001	Add freeze-dried *L. plantarum* ZLP001 to diet	Treatment group supplemented with freeze-dried *L. plantarum* ZLP001	*L. plantarum* ZLP001 significantly enhanced expression of the 6 HDP in the jejunum	Wang et al. (2019c)
1 to 20 d	Landrace × Yorkshire piglets	*L. plantarum* 299v	*L. plantarum* 299v dissolved in 2 mL of 0.1% peptone	Probiotic groups were given *L. plantarum* 299v dissolved in 2 mL of 0.1% peptone daily	Probiotic groups lower diarrhoea incidence, *pBD2, pBD3* and *ZO-1* expression increased in jejunum and ileum	Wang et al. (2019e)
20 ± 2 d	The offspring of L 359 boars mated to Camborough females	*Clostridium butyricum*	Add *C. butyricum* to the pig diet	Add $1,250 \times 10^8$ CFU/kg, $2,500 \times 10^8$ CFU/kg and $3,500 \times 10^8$ CFU/kg *C. butyricum* to the diet of the treatment group	The depth of crypt and the width of villi increase with the dosage of *C. butyricum*to	Casas et al. (2020)
1 to 3 d	Duroc × Landrace × Yorkshire piglets	Fecal microbiota from gestation sows combined with *C. butyricum* and *Saccharomyces boulardii*	Oral administration	FMT-CS group - gestation sows fecal microbiota combined with *C. butyricum* and *S. boulardii*	Early-life intervention significantly increased the alpha diversity of gut microbiota and plasma IL-22 and IL-17	Xiang et al. (2020)
1 to 6 d	Duroc × Landrace × Yorkshire piglets	Maternal FMT	Oral administration	FMT group-3 mL maternal fecal microbiota solution (>10^9 CFU/mL)	Increase of *Blautia* and decrease of *C. sensu stricto* in the relative abundances	Lin et al. (2018)
1 to 2 d	Danish landrace × Large White × Duroc	Colon microbiota suspension of suckling piglets	Oral + rectal administration or rectal FMT	Cesarean delivered preterm pigs were administered combined oral + rectal or rectal FMT	Only rectal FMT increased the stomach-to-colon pH gradient and resistance to mucosa bacterial adhesion	Brunse et al. (2019)
1 to 10 d	Yorkshire piglets	Min sows (an indigenous pig breed in China) fecal microbiota suspension	Oral inoculation	Recipient group: oral FMT; control group: orally inoculated with sterile physiological saline	On d 21, the relative abundance of the Proteobacteria was reduced; the concentrations of IgM and IgG in the jejunal mucosa, and that of IgG in the ileal mucosa of the recipient group, were increased	Teng et al. (2020)

PBS = phosphate buffer saline; sIgA = secretory immunoglobulin A; ZO-1 = zonula occludens 1; FMT = fecal microbiota transplantation; IAA = indoleacetic acid; AhR = aryl hydrocarbon receptor; HDP = host defense peptide; *pBD* = β-defensin; IgM = immunoglobulin M; IgG = immunoglobulin G.

6. Regulation of microflora intervention on intestinal microecology of pigs

In pig production, healthy breeding is critical. Only when pigs have a healthy intestine can they grow efficiently and healthily. A healthy intestine is the sum of a balanced intestinal microecology and mucosal immunity.

6.1. Fecal microbiota transplantation (FMT)

FMT is one strategy to reconstruct the intestinal microecology, and is considered to be a breakthrough in recent years. This technology is not only widely studied in human medicine, but has been applied to pig production as well. An increasing number of studies have been conducted on flora transplantation from donor to recipient pigs. Here, we also list some studies on the effects of microbial intervention on intestinal mucosal immunity in pigs (Table 1). The fecal microorganisms of Chinese local Jinhua pigs were transferred to Duroc × Landrace × Yorkshire commercial piglets by FMT. It was found that the average daily gain of recipient pigs increased, rates of diarrhea decreased, intestinal morphology and physical barrier integrity improved, and the number of goblet cells, *MUC2* expression, and immune-related receptor expression in the intestinal mucosa increased. High-throughput sequencing of 16S rDNA showed that the abundance of Prevotellaceae, Firmicutes, *Ruminococcus* and other microorganisms in the colon increased after transplantation (Hu et al., 2017). In a study by Cheng et al., the expression of 289 proteins in the intestinal mucosa of piglets receiving Jinhua pig FMT changed, and the expression of proteins related to inflammation in the intestinal mucosa decreased. The piglets were then infected with *E. coli* K88. Compared with the piglets that did not receive FMT, the abundance of beneficial bacteria such as *Lactobacillus* and *Succinivibrio* increased, the number of Enterobacteriaceae and Proteobacteria bacteria decreased, and the intestinal morphology and barrier integrity improved (Cheng et al., 2018). Furthermore, FMT enhanced the diversity and composition of colonic flora in piglets, which also resulted in increased levels of the tryptophan catabolite IAA in the colonic lumen, as well as IL-22 and AhR (Geng et al., 2018). Hu et al. transplanted healthy microorganisms from Jiangxiang pig feces to Landrace × Yorkshire piglets, which resulted in a significant reduction in diarrhea attributed to early weaning stress. The authors proposed a potential mechanism of this protection in which *Lactobacillus gasseri* LA39 and *L. frumenti* can secrete bacteriocin or gassericin A. These proteins bind to the host IEC membrane protein keratin 19, which appears to enhance intestinal water absorption, thus exerting the anti-diarrhea effect of FMT (Hu et al., 2018b).

These studies demonstrate the potential efficacy of FMT in pig production, and the important role of intestinal microorganisms in the health of the animals. Therefore, various techniques, including FMT, can be used to screen microorganisms that are beneficial to porcine intestinal health and promote the development of intestinal mucosal immunity. The rational tailoring of intestinal flora can be used to solve the common problems of diarrhea and abuse of anti-biologicals in breeding and production operations, and improve animal health.

The fecal suspensions used by FMT include bacteria, archaea, fungi, protozoa, viruses, cytokines and various metabolites. We do not know which microorganisms or which factors or products play a role in the treatment of which diseases. At present, all the microbiota in the faeces are transplanted across the board, which may not be the best way, and may bring the risk of unknown pathogenic microorganism infection. The selection of donors is particularly important in porcine FMT technology, and different donors may affect the efficacy of FMT technology. Donors must be strictly screened to minimize the risk of potential disease infection by the recipient. In addition, the pig manure suspension contains a large number of microorganisms and impurities, which may affect the efficacy of pig FMT technology. Therefore, how to highly purify fecal suspension without affecting the microbial activity in fecal suspension is worthy of further research and discussion.

6.2. Dietary nutritional composition

In previous research, the research hotspot of pig nutritional feed mainly focused on the regulation of nutrients on physiological function (Li et al., 2019). Along with the development of cross-disciplinary studies, this remains an important means to reshape intestinal health by changing the intestinal flora through nutritional intervention (mainly carbohydrates, amino acids and proteins) to regulate the imbalance of intestinal immunity in pigs.

Dietary fiber is the substrate of microbial fermentation in the pig intestinal tract. The fiber used in pig feed mainly includes corn fiber, soybean fiber and wheat bran. Different types of fiber have different effects on the composition of pig intestinal microflora and short-chain fatty acids (SCFA) due to different water-soluble water binding capacity. Highly diversified fiber diets can induce the increase of bacterial diversity (Van Nevel et al., 2006). Zhang et al. found that alfalfa diet increased the number of *Clostridium* cluster XIVb and *Sporobacter termitidis* in the cecum compared to the pure cellulose diet (Zhang et al., 2016). Long-term intake of fiber (maize fiber, soybean fiber, wheat bran fiber and pea fiber) with different fiber diets has different effects on intestinal bacterial composition and mucosal digestive physiology of (fattening pigs) in fattening pigs. Among them, wheat bran fiber and pea fiber could promote the morphology of porcine intestinal mucosa and the expression of disaccharidase and glucose transporter, while feeding soybean fiber damaged the digestive physiological structure of porcine intestinal mucosa (Chen et al., 2014).

Amino acid metabolism determines the activation and function of immune cells, such as the proliferation of immune cells and the secretion of cytokines, which is closely related to the fate of immune cells (Li et al., 2019). Arginine is the precursor of nitric oxide synthesis, which can promote macrophages and neutrophils to resist pathogen infection. L-arginine can reduce the expression of *TLR4* induced by LPS, inhibit the activation of TLR4/nuclear factor κB (NFκB) and mitogen-activated protein kinase (MAPK) signal pathways, attenuate the inflammatory response induced by LPS, and stimulate the expression of *pBD-1* in porcine epithelium by activating the target of rapamycin (mTOR) pathway in mammals (Lan et al., 2020). Adding 1.0% L-arginine to the diet of piglets could increase the serum insulin concentration and villus height of jejunum and ileum (Zheng et al., 2018). The above research results partially indicate that changes in dietary nutrients can affect intestinal health by changing microbial metabolism.

6.3. Probiotics and prebiotics

The feed industry has prohibited using antibiotics as a feed additive, and people's expectations for probiotic additives are increasing. As a microbial agent, probiotics help maintain the integrity and stability of the intestinal mucosa, the homeostasis of the flora, and promote intestinal immunity. *Saccharomyces boulardii* is widely used to relieve acute intestinal inflammation and acute diarrhea (Badia et al., 2012; Chang et al., 2017). *Lactobacillus* that relieves acute and chronic diarrhea and promotes intestinal development (Lee et al., 2009), and *Clostridium butyricum* can regulate intestinal immunity (Li et al., 2018). Studies have shown that *Enterococcus faecium* Strain NCIMB 10415 can reduce the abundance of *E. coli* virulence factors and reduce the number of

Table 2
Examples of trials regarding the effect of prebiotics on pig.

Age of pig	Breeds of pig	Prebiotics	Main outcome	Reference
2 to 14 d	Offspring of Topigs sows	FOS	Piglets supplemented with FOS had numerically greater villi and deeper crypts, modulates the bacterial colonization of the gut and the intestinal development	Schokker et al. (2018)
21 to 42 d	Offspring of Large White crossbred sows	RPS	RPS intake increases the content of anaerobic bacteria in feces, and the concentration of butyrate in the intestine, and increases the content of regulatory T cells in the cecum	Trachsel et al. (2019)
4 months	Large White male growing pigs	TGS	TGS down-regulated the caecal expression of zonula occludens-1 and mucin 2 and of genes within the toll-like receptor 4 and nuclear factor κB pro-inflammatory signaling cascade	Metzler-Zebeli et al. (2018)
23 ± 2 d	Duroc × Landrace Large × White barrow	YG	YG group up-regulated the expression of occludin m-RNA in the duodenum and jejunum mucosa, increased the relative abundance of *Lactobacillus*, and decreased the acetate content	Qin et al. (2019)
From d 86 of gestation to d 20 of postpartum (sows); 7 to 35 d (piglets)	Landrace × Yorkshire sows and their offspring (Duroc × Landrace Yorkshire)	MOS	sow diet MOS decreased proinflammatory cytokines IL-2 and IL-4 concentrations in piglet serum, Piglets diet MOS decreased the contents of IL-2, IL-4 and interferon γ while increased anti-inflammatory cytokine IL-10 content in serum	Duan et al. (2019)
21 to 35 d	Huanjiang mini-piglets	SBOS	SBOS supplementation increased elevated the numbers of beneficial intestinal bacteria, also increased the concentration of short-chain fatty acids in the intestinal lumen, and it reduced the numbers of bacteria with pathogenic potential (e.g., *E. coli*, *Clostridium*, and *Streptococcus*)	Zhou et al. (2014)
28 d	Duroc × Landrace × Yorkshire piglets	AMSLF	AMSLF supplementation significantly decreased diarrheal incidence in piglets, 7.50% AMSLF group having higher IL-2 and TNF-α levels than the other treatment groups	Che et al. (2019)
First week after birth	Duroc × Landrace × Large White piglets	GOS	On d 8 and 21 after GOS intervention, ileal microbiota composition was significantly enriched in *Lactobacillus* and unclassified Lactobacillaceae, and the expression of anti-inflammatory cytokines and antibacterial peptides increased	Tian et al. (2019)
Approximately 8 weeks	Yorkshire-Landrace pigs	Inulin	Inulin supplementation up-regulated Th2-related immune genes (*IL-13*, *IL-5*), and suppressed Th1-related pro-inflammatory genes (interferon γ, *IL-1A*, *IL-8*) in the colon	Myhill et al. (2018)

FOS = fructooligosaccharides; RPS = resistant potato starch; TGS = transglycosylated starch; YG = yeast glycoprotein; MOS = mannan oligosaccharide; SBOS = soybean oligosaccharides; AMSLF = *Astragalus membranaceus* fiber; GOS = galacto-oligosaccharide.

pathogenic *E. coli* adhering to intestinal mucosa (Bednorz et al., 2013). *C. butyricum* increased the levels of intestinal tight junction proteins (ZO-1, claudin-3 and occluding) in pigs infected with enterotoxigenic *E. coli* K88, decreased the levels of IL-1β and IL-18 in the intestines, and increased the level of IL-10 (Li et al., 2018). The addition of *C. butyricum* and *E. faecalis* to the diet of weaned piglets can improve growth performance, which may regulate intestinal morphology by activating the TLR4-mediated MyD88-dependent signaling pathway (Wang et al., 2019d).

As a substitute for antibiotics, prebiotics are more and more widely used in the field of animal breeding. Prebiotics refers to a class of substances that cannot be digested by the host itself, which can selectively promote the growth or activity of specific bacteria in the intestinal tract, thereby promoting the health of the host, such as functional oligosaccharides, polysaccharides, polyols, protein hydrolysates, and plant extracts (Markowiak and Slizewska, 2018). Oligosaccharide prebiotics such as fructo-oligosaccharides can improve the intestinal mucosal barrier in suckling piglets (Schokker et al., 2018). Resistant starch increases the concentration of butyrate in the colon of weaned piglets and promotes intestinal immunity (Trachsel et al., 2019). At present, there are many studies on the application of prebiotics in pigs. Table 2 lists the experimental examples of the effects of prebiotics on pigs. Supplementation of 1,000 mg/kg chlorogenic acid to basal diet improves the intestinal barrier of weaned piglets by inhibiting the TLR4/NFκB signal pathway and activating the Nuclear factor erythropoietin-2-related factor 2/heme oxygenase 1 (NRF2/HO-1) signal pathway (Chen et al., 2018). Le Bourgot et al. (2017) reported that the addition of short-chain fructo-oligosaccharides to the diet of pregnant sows could increase the secretion of IFN-γ and IL-4 in the ileum of piglets, increase the number of cecal goblet cells and increase the content of IgA in serum and ileal mucosa. Wang et al. showed that feeding galactose oligosaccharides to piglets during lactation increased total SCFA concentration, and promoted the gene expression of inflammatory cytokines (*IL-8* and *IL-10*) and tight junction proteins (*ZO-1* and claudin) in the colon (Wang et al., 2019a). The utilization degree of different prebiotics by pig intestinal flora is different. There are differences in the degree of proliferation of beneficial bacteria and the degree of inhibition of harmful bacteria, and a lot of research needs to be done in its practical application.

The combination of probiotics and prebiotics in the form of synergism is called synbiotics. Synbiotics have the characteristics of both probiotics and prebiotics. Probiotics can promote the balance of intestinal microecology, and prebiotics can provide nutritional substrates for probiotics, thus improving the viability of probiotics in the gastrointestinal tract (Vrese and Schrezenmeir, 2008). Many studies have shown that specific synbiotic combinations can have beneficial effects on the host. For example, the compound addition of probiotics and prebiotics can better improve the intestinal barrier function and health status of pigs. Adding 5% corn husk 10^8 CFU/g *C. butyricum* and can improve the growth performance of weaned piglets and reduce the mortality of piglets. It is further revealed that *C. butyricum* can improve the intestinal health of piglets by optimizing the structure of microbial flora and improving the production and utilization efficiency of acetate and butyrate (Zhao et al., 2018). The combined use of 0.2% *Lactobacillus plantarum* ZLP001 and 0.5% FOS in the diet of piglets could significantly increase the abundance of *Lactobacillus*, reduce the number of Enterobacteriaceae, and increase the concentration of serum INF-γ and IgG, showing a good synergistic effect (Wang et al., 2019b).

In the preparation of synbiotics, the selection of probiotics and prebiotics and their effects on intestinal microorganisms are very important. There are great differences in the methods, doses and

duration of symbiotic studies. At present, it has gradually become a convenient method to study intestinal microflora and their metabolites to evaluate the effect of synbiotics by simulating the intestinal environment by fermentation in vitro.

7. Future prospects and challenges

Intestinal flora contributes to the development of and regulation of the intestinal mucosal immune system. Evidence also suggests that gut flora also plays a direct protective effect against pathogenic bacteria. At present, only a small number of intestinal bacteria have been studied with regards to their effects on the differentiation of immune cells. The data on most microorganisms are not clear about the differentiation of immune cells. In addition, the intestinal microflora also includes viruses, phages and eukaryotic microorganisms. It is well established that these organisms co-evolve with the host immune system, and the interaction between them and the immune system requires further in-depth analyses. Beyond interactions with the host immune system, in-depth studies on metabolic utilization pathways, immune regulation, and regulation of gene expression by intestinal flora through modern nutrition are likely to be hot fields of future discovery. Understanding the different types of intestinal microorganisms and their regulatory mechanisms, especially those between intestinal microflora and mucosal immunity, is of great significance to understanding optimum nutrition for various livestock industries, particularly pig production.

According to existing literature reports, there are still some challenges to promoting mucosal immunity through intestinal microorganisms. First of all, living intestinal microorganisms are different from any drug, and their particularity comes from the fact that the bacteria themselves are living cells. In the actual production and application, how many live bacteria can reach the intestinal tract? How can we better maintain the number of living bacteria by changing the dosage form, or adding some protective agents or microbial coating technology, so as to promote the colonization of microorganisms in the intestinal tract? In addition, with the application of microfluidic chip technology in biomedicine, the construction of the pig intestinal system in vitro plays an important role in realizing the cultivation of pig intestinal flora and studying the influence of different microorganisms and metabolites on intestinal mucosal immunity (Jalili-Firoozinezhad et al., 2019; Shah et al., 2016).

Author contributions

Yanhua Huang: conceptualization, review. **Jie Peng**: conceptualization, investigation, review. **Yimei Tang**: conceptualization, data curation, formal analysis, visualization, writing.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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