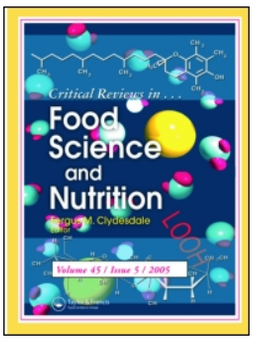

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Interaction between gut immunity and polysaccharides

Xiaojun Huang, Shaoping Nie, and Mingyong Xie

State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, P. R. China

ABSTRACT

The human gut is colonized with a vast and diverse microbial ecosystem, and these bacteria play fundamental roles in the well being of our bodies. Gut-associated lymphoid tissues, the largest mucosal immune system, should never be overlooked for their profound effect in maintaining the host immunity. Therefore, we discussed the relationship between gut immunity and host health, primarily from two aspects: the homeostasis of gut microbiota, and the function of gut-associated lymphoid tissues. Polysaccharides, widely concerned as bioactive macromolecules in recent centuries, have been proved to benefit the intestinal health. Dietary polysaccharides can improve the ratio of probiotics, regulate the intestinal microenvironment like decreasing the gut pH, and stimulate the macrophages or lymphocytes in gut tissues to fight against diseases like cancer. Based on various experimental and clinical evidence, the impacts of dietary polysaccharides on intestinal health are summarized, in order to reveal the possible immunomodulatory mechanisms of polysaccharides.

KEYWORDS

Gut microbiota; gut-associated lymphoid tissues; polysaccharides; inflammatory bowel disease

Introduction

Polysaccharide is a kind of biological macromolecule widely distributed in nature. In 1988, Dwek RA from University of Oxford published a review named "Glycobiology," which symbolized the birth of glycobiology (Rademacher et al., 1988). Over the past decades, numerous polysaccharides derived from nature have been proved to have significant anti-inflammatory (Komura et al., 2010), anti-cancer (G. Li et al., 2014), anti-tumor (Liu et al., 2015), and anti-oxidant activities (Xing et al., 2013; Ren et al., 2015). Recently, the biological benefits of polysaccharides on gut immunity have attracted attention among researchers. In 2008, a review published in *Nature Reviews Microbiology* (Flint et al., 2008) provided a new insight into how polysaccharides were utilized by gut bacteria. Ochoa-Reparaz and coworkers (2010) reported the protective effect of commensal *Bacteroides fragilis* polysaccharide A against CNS demyelinating polysaccharide, which leads to a specific attention on the role of polysaccharides in human health. Subsequently, another study (Sims et al., 2011) into the structure and functions of *Lactobacillus reuteri* exopolysaccharide was reported next year. In 2012, another review published in *Nature Reviews Microbiology* (Koropatkin et al., 2012) discussed deeply into the molecular mechanism of how glycan metabolism shapes gut microbiota. In 2014, a study (Larsbrink et al., 2014) published in *Nature* demonstrated a discrete genetic locus in *Bacteroides ovatus* for xyloglucan catabolism; and another article in *Current Biology* offered a complex ecological network of polysaccharides utilization by intestinal symbionts based on experimental evidence (Rakoff-Nahoum et al., 2014). They also promote the research of the polysaccharide' function in gut immunity into a higher level. Therefore, it is not difficult to judge that study on the relationship between polyacchride and gut immunity has become a hotspot in scientific research.

In addition, the study of immunomodulatory mechanism of polysaccharides from the view of gut immunity is accepted. Most polysaccharides are nondigestable in human intestine (Flint et al., 2008), but can be fermented and utilized by gut microbiota or interacted with the immune cells in gut-associated lymphoid tissues (GALT). On the one hand, the gut microbiota carry out substantial roles in maintaining host physiology, such as defining the maturation of immune system (Blaut and Clavel, 2007), responding in epithelial cell injury (Kinross et al., 2011), participating in the energy metabolism (Clemente et al., 2012), and inhibiting the growth of potential pathogens (Wallace et al., 2011). Disorders in adult gut microorganisms are involved in a large number of disease, including obesity, type 2 diabetes, allergy, intestinal bowel disease, and even HIV progression (Fujimura et al., 2010). On the other hand, GALT is the largest lymphoid tissues collection in body, heavily laden with macrophages, dendritic cells (DCs), and lymphocytes involved in immune response. The formation of a certain disease results from various challenges from the outside environment (Faubion, 2013), and these challenges are, in particular, cases of digestive tract. Based on these facts, what happens in gut for polysaccharides is of great importance for exerting its biological activity. Therefore, the study of the relationship between polysaccharides and gut immunity can provide us new insights in discussing the biological effects of naturally derived polysaccharides.

Inflammatory response is beneficial in repairing the damage in body; however, excessive inflammaton will cause adverse effects to organisms. Accumulating clinical studies have

CONTACT Shaoping Nie spnie@ncu.edu.cn State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, P. R. China.
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revealed that many carcinogenesis diseases are derived from long-term chronic inflammation (Liang et al., 2014). Colorectal cancer is one of the diseases developed from the establishment of chronic inflammation in colon (Liang et al., 2014). It is reported that people with inflammatory bowel disease (IBD) are at higher risk of getting colorectal cancer (Liang et al., 2014). The development of IBD is accompanied with the dysbiosis of gut microbiota and damage in mucosal lymphoid system (Faubion, 2013). However, as mentioned before, the consumption of polysaccharides can influence the gut microbiota composition and mucosal lymphoid tissues. Consequently, dietary polysaccharides are called for the treatment of IBD, instead of therapeutic drugs with serious side effects.

In this review, we give an overview of the roles that gut microbiota and GALT play in individuals' health: how imbalances in the composition of the microbiota relate to diseases, what factors can alter the microbiota ecosystem; and how immune response is induced to infection. Moreover, the mechanisms for the therapeutical effects of phytochemical, polysaccharides, on IBD are also discussed.

Gut immunity and human health

Role of the gut microbiota in gut immunity

The human gastrointestinal (GI) tract carries more than 1014 microbial cells with more than a thousand diverse types (Kinross et al., 2011; Wallace et al., 2011). The densities of these microorganisms increase from stomach to colon and they play a fundamental role in host health. The term, gut microbiota, is given to describe the microbial associates that populated in digestive system of animals and humans (Kinross et al., 2011; Clemente et al., 2012). And the term, microbiome, referred to the genes they encode (Clemente et al., 2012). In most mammals, gut microbial communities are mainly dominated by four main bacterial phyla, *Firmicutes, Bacteroidetes, Actino* bacteria, and *Proteo* bacteria (Kinross et al., 2011; Xu et al., 2013). The interindividual differences of microbial composition are more marked in infants than adults, but later in life the phylotype composition becomes similar (Blaut and Clavel, 2007; Kinross et al., 2011; Clemente et al., 2012). Once the microbiota structure has reached its maturity, it remains stable until old age (Clemente et al., 2012). However, this temporal stability in microbial composition can still be varied by diet, disease, environment, et al. (Clemente et al., 2012; Xu et al., 2013). The gut microbiota perform a vast number of important roles that define the physiological function of host, for instance, influencing the development and maintenance of immune system, promoting the cell proliferation, as well as contributing to a series of metabolic actions such as the nutrients absorption and energy storage. Besides, the gut microbiome also plays an essential role in determining the toxic response to medical therapies. Based on its various functions to organism's health, gut microbiome can provide fertile messages for the development of new generation of therapeutic drug targets (Kinross et al., 2011).

The analysis of the effects of gut microbiota on both intestinal mucosal and systemic immune systems is readily appreciated due to the advent of germ-free (GF) animals (Sekirov et al., 2010). Another pivotal technique, 16S rRNA gene-sequence-based method, reveals the description of intestinal microbiome diversity among individuals (Turnbaugh et al., 2007). However, this method still has some limitations. The interactions between symbiotic gut flora and intestinal barrier is complex, they may occur at any interfaces, yet fecal microbiome cannot represent the ecosystem variations at all levels. Yet this analytic method for fecal samples cannot provide a comprehensive message of bioactive molecular interactions within the topography and niches in GI tract (Kinross et al., 2011).

Factors that shape the gut microbial community

The intestinal microbial community is correlated with other factors, such as host genetics, diet, age, the abuse of antibiotics, environment, and so on.

Effects of host genotypes on gut microbial community. Each host has a specific biological relationship with its enterotypes; in other words the gut microbiota community is also host genetic-unique (Kinross et al., 2011). Wen et al. (2008) found that the knockout of MyD88 gene could protect the non-obese diabetic (NOD) mice from type 1 diabetes. And this result was due to the difference of microbiota diversity between MyD88$^{+/-}$ NOD mice and MyD88$^{-/-}$ NOD mice, with more *Porphyromonadaceae, Lactobacillaceae*, and *Rikenellaceae* phylotypes observed in MyD88$^{-/-}$ NOD mice. Khachatryan et al. (2008) studied the difference of gut microbiota diversity between amilial Mediterranean fever (FMF) patients with Mediterranean fever (MEFV) gene mutation and healthy individuals. The results indicated that the FMF patients were characterized by a decrease in gut microbiota number and diversity, as well as a variation in the ratio of *Bacteroidetes, Firmicutes*, and *Proteobacteria* phyla. At the gene level, the microbiome that are identified as functional genes are regarded as "core microbiome." The changes in these functional "core microbiome" are relevant to the difference of host phenotypes (Xu et al., 2013). Another classical argument about the relationship between gut microbiota structure and host genotypes is the hypothesis of microbiome heritability. Studies on the microbiome of monozygotic (MZ) and dizygotic (DZ) twins have been made to explore the facticity of microbiome heritability (Spor et al., 2011). In an earlier study (Stewart et al., 2005), a slightly higher within-pair similarity of microbiota in MZ twins was discovered compared with that in DZ twins, supporting the heritability of gut microbiota. However, a study in 2009 (Turnbaugh and Gordon, 2009) worked out a contrary result. Therefore, the heritability of gut microbiota was still under hot discussion (Spor et al., 2011). In the latest study in 2014, Goodrich et al. (2014) analyzed the microbiota diversity of fecal samples from 171 MZ twins and 245 DZ twins, and found that the operational taxonomic units' (OTU) relative abundance are more correlative in the former. Meanwhile, in an animal study between mouse lines, the effects of host genotypes on microbiome diversity were identified. Benson et al. (2010) found that the host genetics had a considerable contribution to the distinction of gut microbiota community between C57BL/6J mice and ICR-derived outbred mice.

Effects of age on gut microbial community. It is well acknowledged that aging process has distinct impacts on human microbiota community in gut and host immune system (Xu et al., 2013). In general, we are considered sterile in utero, which can be verified by rare viral particles and low diversity of bacteria in meconium (Clemente et al., 2012). The microorganisms in infants can get the functions found in mothers. Infants are exposed to microbes from different environments promptly since birth, and are resided in with microbiotas they first encounter (Clemente et al., 2012). Those born vaginally obtain the enterotypes from their mothers' vagina; while those born by caesarean have a community characteristic of skin mainly consisting of taxa like *Staphylococcus* and *Propionibacterium* spp. Additionally, the delivery modes are also assumed to affect the babies' immune functions in the first year of their lives, showing that caesarean born baby has lower bacterial counts in feces and more antibody-secreting cells (Turnbaugh et al., 2008). In young adults, the gut microbiota is primarily dominated with *Firmicutes* and *Bacteroidetes*, over 90% in abundance, and the *Firmicutes* are higher than *Bacteroidetes* in feces (Mariat et al., 2009). In elderly adults, the composition is different. Compared with those in young subjects, the dominant microbes switch from *Firmicutes* to *Bacteroidetes* in fecal samples of the older, and this variation is hypothesized to make up for their degenerative digestive system. On the contrary, the diversity of species in *Bacteroidetes* was decreased in elderly, with lower proportion of *Bifidobacterium* (Xu et al., 2013). In centenarian, the diversity of microbial communities is even lower. The centenarian intestine is populated with an increasing *Eubacterium limosum* and decreasing *Faecalibacterium prauznitzii*. *F. prauznitzii*, however, is considered to have anti-infiammatory ability. Therefore, it is reasonable to explain the high inflammation status in centenarians (Biagi et al., 2010). The effects of age on gut microbiota diversity have been summarized in Fig. 1 (Mariat et al., 2009; Clemente et al., 2012).

Effects of diet on gut microbial community. "Your gut microbiota are what you eat!" Chewapreecha's (2014) announcement brought us the message that the dietary variations can greatly lead to the shifts in gut microbiota community. It is known that the microbiome of a healthy adult is relatively stable once it is established, with a minimal change in over 95% viral sequences in a one-year period (Clemente et al., 2012). However, it is proved that the microbiomes of healthy human individuals can converge to a more alike status resulting from dietary intervention (Clemente et al., 2012). Experiments in mice also present similar results. The mice fed with a high-fat diet have more *Firmicutes* than *Bacteroidetes*; in other words, the ratio of *Firmicutes*/ *Bacteroidetes* is correlated with increasing energy harvest from diet (Hildebrandt et al., 2009). Additionally, diet can also change the enterotypes, for individuals with high-fat food have a *Bacteroides*-dominated enterotype, while a polysaccharide-rich diet are colonized with a *Prevotella*-dominated enterotype (Clemente et al., 2012). Another investigation in infant diets showed that the GI tract of breast-fed infants is mainly housed with *Bifidobacteria* bacteria, whereas the *Bacteroides* and *Clostridium* coccoides are dominant in feces of formula-fed infants (Harmsen et al., 2000).

The nutritional ingredients in diet also have complex impacts on intestinal microbiota community (Xu et al., 2013). Dietary nutrients, such as carbohydrate, protein, and fat, can be digested by host enzymes and absorbed in small intestine. However, still some remaining parts, such as nondigestible carbohydrate and residual protein, have escaped the digestion and

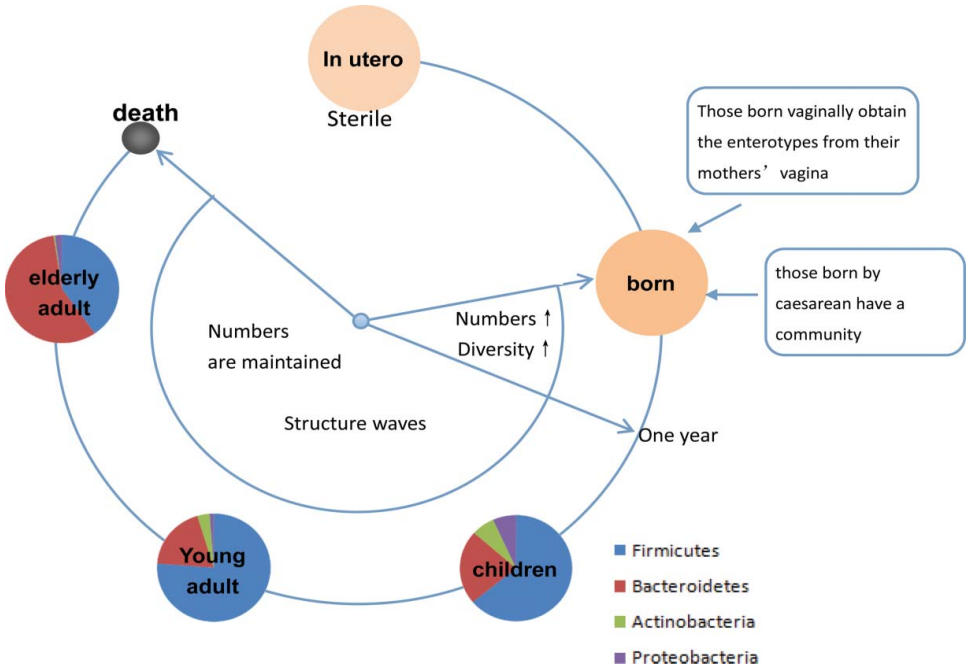

Figure 1. Variations of gut microbiota with ages. In utero, we are considered sterile. Infants are resided in with microorganisms they first encounter. Those born vaginally obtain the enterotypes from their mothers' vagina, while those born by caesarean have a community characteristic of skin. From children to young adult, the proportion of *Firmicutes* in gut microbiota is increasing, while the proportions of *Bacteroidetes, Actinobacteria*, and *Proteobacteria* are decreasing. From young adults to elderly adults, these variations shift, with the dominant microbes switching from *Firmicutes* to *Bacteroidetes* in feces, while the proportions of *Actinobacteria* and *Proteobacteria* remain decreasing. The number of gut microbiota significantly increases in the first year when we were born, but later remain stable till death. Similarly, the diversity of microbiota increases in the first year when we were born; however, in the later years, the structure of microorganism waves.

reached the colon (Flint et al., 2008). On the one hand, these materials can provide specific nutrients to certain bacterial species, therefore promoting the growth of specialized microbiota (Rakoff-Nahoum et al., 2014). On the other hand, these components will be fermented into short-chain fatty acid (SCFA) and other metabolites, by gut microbiota. And such metabolites can change the gut environment, including moisture and pH, which remarkably impact the growth of different microorganisms in GI tract (Hu et al., 2012). Take dietary protein for example: the protein that remains in the colon can provide nitrogen and amino acids for the growth of *Saccharolytic* species and *Asaccharolytic* species, respectively. *B. Fragilis* group, *Clostridium perfringens, propionibacteria, streptococci, bacilli*, and *staphylococci* are main proteolytic bacteria (Scott et al., 2013; Xu et al., 2013). The fermentation of amino acids happens in distal colon where polysaccharide polymers are depleted and the environmental pH is near neutral. Whereas *Bacteroides* species have a strong peptidase activity even when its environmental pH is around 6.5 (Walker et al., 2005). The dietary fat can be digested and absorbed mostly in small intestine, but recent studies showed that ^{13}C marked fatty acids were found in stool. Usually, a high-fat diet is accompanied with low dietary fiber intake in order to construct energy equivalent diets, which results in reduced SCFA, therefore leading to the variation of gut colonizers (Scott et al., 2013).

Other factors affecting gut microbial community. Besides the above-mentioned factors, there are still other factors that can impact the structure of gut microbiota. Regional disparities combined with environmental differences are possible to change the microbial ecosystem of gut in genus and species levels. Americans have higher proportions of *Firmicutes*, Japanese show higher proportions of *Actinobacteria*, and people from China and Korea are dominated by *Bacteroidetes* (Candela et al., 2012). Domestic relation is also considered as a factor for the differentia in gut bacteria. Parents and their offspring, as well as twin sisters/brothers, share a similar microbial community in fecal samples, compared with unrelated individuals (Xu et al., 2013).

Disease and gut microbiota

The host immune system, especially the mucosal immune system, has a close relationship with our GI microbes (Clemente et al., 2012). Numerous studies unveiled that the development of host immune system is affected by gut microbiota (Wallace et al., 2011). Maternal exposure to environment during pregnancy plays a crucial role in the formation of kid's postnatal immune function, especially in the subsequent development of allergy (Fujimura et al., 2010). One of the examples to be mentioned is that mothers exposed to farm work or animals during pregnancy can diminish the probabilities for their children to get allergies and asthma (Fujimura et al., 2010). After birth, the immatured immune system begins to develop upon exposure to gut microbiota (Wallace et al., 2011). In addition, microbial interactions are involved in the development of both the innate and adaptive immune systems (Clemente et al., 2012).

Metabonomics analysis of urine samples also demonstrated the importance of gut microbiota dynamics over time or variations among individuals in disease development and drug metabolism (X. Xu et al., 2013). Recent studies indicated that the etiopathogenesis of certain diseases or disorders, such as diabetes, obesity, IBD, allergy, and autism, are often associated with the dysbiosis of bacterial community. Compared with healthy individuals, the type 2 diabetes patients exhibited an increase in *Bacteroides* versus a decrease in *Firmicutes* and *Clostridia*, and the shifts in intestinal microbiota were correlated with increase in plasma glucose concentration (Clemente et al., 2012). For obese patients, a remarkable 10-fold increasing in the ratio of *Firmicutes*/*Bacteroidetes*, from 3:1 to 35:1, was observed, compared with lean subjects (Fujimura et al., 2010). A complicated dysbiosis of gut microbiota was discovered in the IBD process, with the increased proportion of *Actinobacteria* and *Proteobacteria* and decreased proportion of *Bacteroidetes, Lachnospiraceae, Clostridium, Faecalibacterium*, and *Bifidobacteria* (Clemente et al., 2012). The development of allergy is reported to accompany with observed drop in *Lactobacillus* spp., *Bifidobacterium adolescentis, Clostridium difficile*, and *Helicobacter pylori*. An investigation in the feces of autistic children indicated a mount in bacterial diversity (Robinson et al., 2010). Based on these evidence, the maintenance of microbial community plays an importance role in human health; indeed, it is regarded as our "forgotten organ" to some extent (Clemente et al., 2012).

Role of GALT in gut immunity

The structure and composition of GALT

Mucosal immune system, also described as mucosa-associated lymphoid tissue, is widely distributed in the mucous membranes of tissues of respiratory tract, GI tract, and urogenital tract. It is the primary place for the initiating of specifc immunity. GALT, one part of mucosal immune system in GI tract, is the largest collection of lymphoid tissues in the body (Forchielli and Walker, 2005). It can be divided into initiation sites and effector sites. The initiation sites are composed of Peyer's patches (PPs), mesenteric lymph nodes (MLNs), isolated lymphoid follicles (ILF), and crytopatches. While the effector sites consist of lymphocytes scattered throughout the intraepithelial lymphocytes (IEL) and lamina propria (LP) of the mucosa.

The PPs are macroscopic lymphoid aggregates located in the submucosa along the small intestine (Mowat, 2003). The lymphoid areas are covered with a single layer of specialized epithelial cells (ECs), to separate from the intestinal lumen. This single layer is named the follicle-associated epithelium (FAE) (Forchielli and Walker, 2005). The FAE is different from the villus epithelium, with less digestive enzymes and brush border (Kunisawa et al., 2012). Additionaly, FAE contains a specific enterocyte, named membranous cell (M cell). The M cell has short microvilli, a thin mucus layer, and small cytoplasmic vesicles, and it can efficiently transfer the antigens from the intestinal lumen into the lymphoid areas of PPs (Forchielli and Walker, 2005; Kunisawa et al., 2012). Therefore, the specialized function for M cells is to bind aggressive pathogens or other particulate antigens (Goodrich et al., 2014). Under the FAE, there is a more diffused area referred to as subepithelial dome (SED) with abundant DCs exiting here. Under the SED area, there are large B-cell follicles and surrounding T cells located in the interfollicular region. The DCs in SED immediately take up

the antigens bound by M cells, then produce and submit the antigenic peptides to T and B lymphoid cells to initiate the immune responses (Mowat, 2003). The mature PPs are infiltrated by a large number of immune cells, among which 75% are B cells, 20% are T cells, and the rest are macrophages and DCs. PPs are regarded as one of the largest organized lymphoid tissues in mucosal immune system. It is said there are hundreds of PPs in humans, whereas 8–10 in mice intestine (Mowat, 2003; Kunisawa et al., 2012).

MLNs are connected with PPs by lymphatic drainage, and they are considered as the largest lymph nodes in the body. When MLNs encounter with antigens, the antigen presenting cells in other parts of the intestine will migrate to MLNs, and recognize and stimulate the initial T cells to exert the immunomodulatory function (Mowat, 2003). IEL is found in the epithelial layer of intestinal mucosa. When encountering antigens, they immediately release cytokines and cause apoptosis of the infected target cells.

The role of GALT in immune response

The mucosal immune system forms about 50–60% of the body's total immune system, and processes approximately 7% of the antibody. Therefore, gut-associated lymphoid tissue, one of the key parts in mucosal immune system, is no doubt of great importance in systemic immune response. Normally, antigens are transported from the lumen to the PPs by M cells. DCs that are located underneath FAE take up the antigens and migrate to T cell region. In the PPs, the DCs sensitize the naive T and B cells to these antigens. And the sensitized T and B cells later are transferred to the MLNs to become mature and get proliferation. These matured cells then enter the systemic circulation through the thoracic duct, and are distributed to submucosal sites of respiratory tracts or intestines. The process is shown in Fig. 2 (Mowat, 2003; Fukatsu and Kudsk, 2011). In these sites, under the impact of helper T cell subtype 2 (TH2) cytokines, such as IL-4, IL-13, and probably others, the production of antibody is promoted. And the B cells that are transformed into plasma cells are able to produce immunoglobulin M (IgM) and immunoglobulin A (IgA), and transport these immunoglobulins across the mucosa through a special mechanism (Kunisawa et al., 2012). In addition, the plasma cells can release dimeric IgA, which can bind to molecules like polyimmune globulin receptor (pIgR) that exist on the surfaces of the mucosal cell. The mucosal cell conveys this pIgR–IgA complex compound to the luminal surface to release the IgA into the lumen. A small proportion of pIgR–IgA complex remaining in the lumen is identified as secretory-immunoglobulin A (sIgA). sIgA does not activate the complement system, but it plays a part by binding to bacterial surface antigens to keep the bacteria from attaching to the mucosa (Mantis et al., 2011; Kunisawa et al., 2012).

Despite the physical barriers, antimicrobial peptides, like defensins generated by ECs and Paneth cells, are further additional barriers. In addition to these barriers, sIgA, which is mainly produced at intestinal mucosa, helps to construct the immunological defense system (Kunisawa et al., 2012). sIgA acts as the first line of defense against the enteric toxins and pathogenic microorganisms. It maintains the mucosal homeostasis in GI tract by preventing the access of antigens and pathogenic microorganisms to epithelial receptors, and promotes its removal by peristaltic and mucociliary activities. In addition, sIgA can also affect the intestinal microbiota composition, decrease the proinflammatory responses, and facilitate the retro-transport of antigens from the epithelium to dendritic cell subsets in GALTs. The reasons for the multiple abilities of sIgA are largely due to its intrinsic complexity, especially the diverse glycan arrays on both polymeric IgA and bound SC (Mantis et al., 2011).

Gut microbiota and GALT

The intestinal mucosa is the largest surface area contacting with the antigens of the outside environment, and the abundant

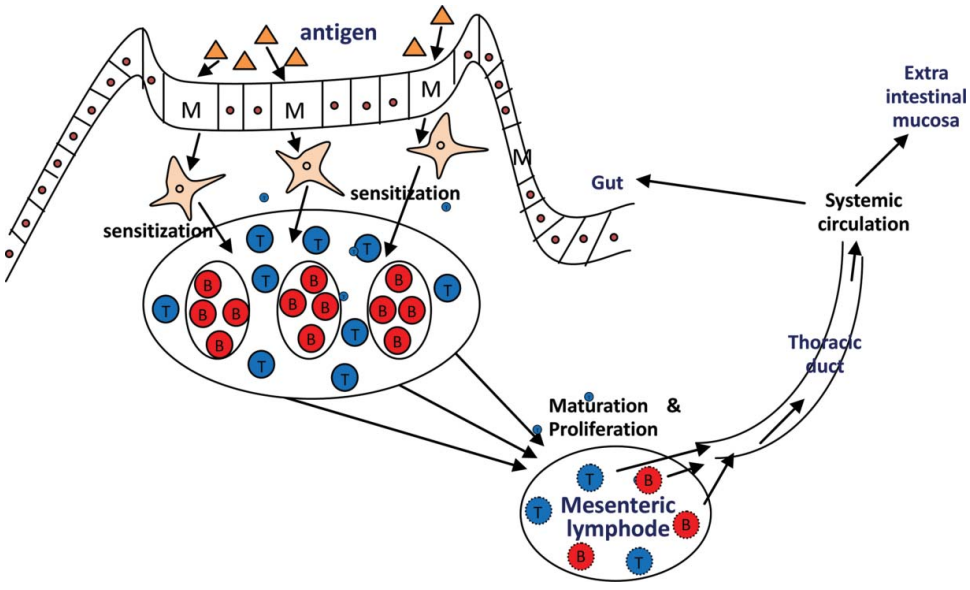

Figure 2. The role of GALT in gut immunity. Antigens are transported from the lumen to the PPs by M cells. DCs that are located underneath FAE take up the antigens and migrate to T cell region. In the Peyer patches, the DCs sensitize the naive T and B cells to these antigens. The sensitized T and B cells later are transferred to the MLNs to become mature and get proliferation. These matured cells then enter the systemic circulation through the thoracic duct, and are distributed to the gut or other extra-intestinal mucosa.

bacterium colonized in the mucosa usually accounts for the biggest portion of the antigens presented to the immune cells in intestinal mucosa or sensitized to the pattern recognition receptors in the ECs (Sekirov et al., 2010). Therefore, it is not surprising that intestinal microbiota plays a vital role in the development of mucosal immunity. Evidence from both animal experiments and human investigations have reinforced the crucial role that the intestinal residents play in the development of GALT (Fujimura et al., 2010). For example, one of the major immune deficiencies in GF animals is the expansion lack in CD4 T-cell populations. However, this deficiency can be totally reversed after being treated with polysaccharide A of *Bacteroides* fragilis. Another good example is that numerous *Lactobacilli* spp. are reported to regulate DCs differentially, followed by impacts on the balance of Th1/Th2/Th3 cytokine in intestinal mucosa (Geuking et al., 2011), and the activation of natural killer (NK) cells. In addition, the mucosal immune system has two effects on gut microbiota: first, it needs to be tolerant to the microbiota in order to inhibit the production of excessive systemic immune response; second, it should be able to control the gut bacterium in order to prevent their overgrowth and translocation in systemic immune system (Sekirov et al., 2010).

The immune system is immature at birth and develops right after the exposure to the gut microbiota (Wallace et al., 2011). It is revealed that the developments of both the innate and adaptive immune systems are evolved with the interactions of gut microbiota. The innate immune system sensitizes the concrete microbiota community generally by microbe-associated molecular patterns (MAMPs) that are present across the lineages of bacteria, such as the flagellin or components in the bacterial cell wall (Clemente et al., 2012). Toll-like receptors (TLRs) are one of the proteins that are employed by host to recognize such antigens. If TLRs are immature or not present, the intestinal mucosal immune system do not construct normally. The commensal microbiota can help in preventing inflammatory response and sitmulating immunological tolerance through TLRs (Round et al., 2011). NOD-like receptor (NLR) is another protein that is used to recognize microbial molecules and can form oligomers to act as sensors of damage-associated patterns (Wells et al., 2011). In these ways, the innate immune system of the host could sensitize the microbial environment, thus stimulating the release of signaling molecules to initiate the immune response. The adaptive immune system is also influenced by the commensal bacteria. It is reported that the microbes have the influence on the differentiation of T cell populations. An example is that mice mono-colonized with a murine segmented filamentous bacterium, presented induction of $CD4^+$ T-helper cells releasing Th17 cytokines (Fujimura et al., 2010).

The commensal microbiota is a kind of bacteria, but unlike the pathogenic bacteria, it peacefully colonizes in the host GI tract. In other words, the host immune system is able to discriminate between the commensal microbiota and pathogens. Therefore, it is not hard to state that the commensal microbiota can influence the development of immune defense system. One way for commensal microbiota or its metabolites to activate the immune response is by restoring cell signaling through innate signaling receptors, e.g., TLRs and NLRs (Round et al., 2011). For instance, TLR4 is involved in the increase of bactericidal lectin RegIIIγ in antibiotic-induced normal microbiota reducing mice with the administration of LPS (Fujimura et al., 2010). NOD1 is engaged by microbiota-derived peptidoglycan to restore the pathogen killing ability of neutrophil (Fujimura et al., 2010; Wells et al., 2011). Additionally, the expressions of enterocyte apoptotic and proliferation genes can also be impacted by commensal bacteria. The complicated microbiota colonization in colon can increase the proliferative area and depth of crypt in colonic epithelium. Therefore, the homeostasis of commensal microbiota is significant in keeping the integrity of intestinal epithelial barrier (Wallace et al., 2011).

Since intestinal microbiota can affect the host immune system, a kind of host beneficial bacteria, probiotics, is applied to further enhance the host immunity. Probiotics can affect the epithelial barrier function in several ways, including influences on the epithelial tight junction proteins, prevention of epithelial apoptosis, promotion of intestinal mucus secretion, stimulation of mucosal secretory IgA, and enhancement of other defensive production (Wallace et al., 2011). In addition, probiotics could also prevent the growth and invasion of pathogens through the following ways: compete with the pathogens for nutrients; ferment the undigested carbohydrate to produce short-chain fatty acids, therefore decreasing the intestinal pH to suppress the growth of pathogens (Flint et al., 2008).

Polysaccharides and gut immunity

Effects of polysaccharides on intestinal microbial community

Polysaccharide is a kind of bioactive macromolecule, distributed in a wide range of sources, like plant, fungi, yeasts, and so on. It is proved by various evidence in vitro and in vivo that polysaccharides have a series of bioactivities, including immunomodulating (Yu et al., 2014), anti-tumor (Zhang et al., 2014), anti-cancer, and anti-oxidant activities (J.-E. Li et al., 2014), kidney protective effects (Nie et al., 2013), colon health benefit effects (Hu et al., 2012), alleviating the obesity and diabetes (Zhu et al., 2014), etc. Also it is acknowledged that the bioactivities of polysaccharides are well maintained after oral administration. However, it is difficult for these macropolymers to be digested totally in GI tract for absorption, or to be absorbed directly by intestine (Flint et al., 2008). Therefore, how orally administered polysaccharides exert their functions has long been a question in the polysaccharide researches.

The extremely dense microbial communities that populate in mammalian GI tract play a crucial role in the utilization of dietary polysaccharides (Spor et al., 2011). The carbohydrate active enzymes (CAZymes) are not encoded in human genome, but exit in the genes of bacterium. It is reported that about 156 CAZymes have been found within one microbiome in human intestine, which indicated a large number of polysaccharide-utilized microbiota in human gut (El Kaoutari et al., 2013). Thus the fermentation of the dietary carbohydrates by gut microbiota is a quite important way for polysaccharides to be used by host. The dietary carbohydrates first break down to oligomeric compounds, and later get further fermentation by gut bacterium into SCFA (Hu et al., 2012), primarily acetate, propionate, and butyrate in a ratio of 3:3:1, as well as CO_2 and

H_2 (Blaut and Clavel, 2007). Lactic acid, ethanol, succinic acid, and formate are other intermediates that will also be degraded into SCFA, CO_2, and H_2 (Blaut and Clavel, 2007). Acetate can enter the systemic circulation and is involved in lipogenesis. Propionate is transported to liver and used in gluconeogenesis. Butyrate, however, is regarded as the main energy source for colonocytes (Scott et al., 2013). Additionally, polysaccharides fermentation and SCFA production can also remarkably promote the absorption of calcium, magnesium, and phosphorus (Wallace et al., 2011).

Nevertheless, the polysaccharides that reach the human gut will influence the intestinal microbial ecology too. And different kinds of polysaccharides have different effects on microbiota structure (X. Xu et al., 2013). A trial on human indicated that fructo-oligosaccharides could stimulate numbers of bifidobacteria, especially *B. adolescentis* and *F. prausnitzii* (Flint et al., 2008). In colonized human fecal microbiota in GF mice, the administration of fructooligosaccharides could result in an increased ratio of *Roseburia* to *E. rectale* group, and a promotion in butyrate levels (Flint et al., 2008). Galacto-oligosaccharides are important component in human breast-milk. The experimental evidence showed that galacto-oligosaccharides supplementation could stimulate the population of *B. adolescentis, Bifidobacterium catenulatum*, and *F. prausnitzii* (Scott et al., 2013). However, similar investigation in elderly people found that this bacteria shift was not totally tested in all individuals (Walton et al., 2012). Arabinoxylans are reported to have the potential ability in stimulating bifidobacteria population and promoting the production of propionate (Pastell et al., 2009). Other animal trails also indicated an alteration of particular microbiota numbers, showing increases in *Bifidobacterium* spp., *Bacteroides-Prevotella* spp, and *Roseburia* spp., as well as advantages on lipid and cholesterol accumulation (Scott et al., 2013). Additionally, the chain length of arabinoxylanoligosaccharides was said be involved in determining the fermentation sites. β-glucan therefore has been proved to influence the diversity of gut bacteria. Oat β-glucan was reported to improve the populations of *Bifidobacteria* and *Lactobacilli*, as well as the butyrate levels in pigs. And the increases in *Bifidobacteria* and *Bacteroides* were also observed in older healthy human volunteers with barley β-glucan diets. Various other studies also indicated that high intake of β-glucan could result in high SCFA and lactic acid concentration. And it is reported that the production of butyrate from the carbohydrate consumption is increased in the order of pectin in batch cultures, shredded wheat, oat xylan, and amylopectin, and pectin in continuous cultures inulin, shredded wheat, oat xylan, and amylopectin (Xu et al., 2013).

The different structural characteristics of polysaccharides also show distinct effects on intestinal microbiota community. The glycosidic linkage type and the molecular size of the polysaccharides are said to impact the profiles of intestinal microbial communities (Sonnenburg et al., 2010).

Different microbiota species have distinct ability in the utilization of polysaccharides. Some of them can ferment a wide range of polysaccharides, while others are substrate specific (Flint et al., 2008). For example, the starch utilization system is said to be abundant and conserved in the phylum *Bacteroidetes*; and *Prevotella bryantii* is efficient in breaking xylan (Scott et al., 2013).

Influences of polysaccharides on gut-associated lymphoid tissues

Evidence worldwide indicated that a large number of polysaccharides derived from natural herbs, fungi, or yeast had the capability to restore the damage in intestinal mucosa, therefore being applied in the therapy of IBD (Hur et al., 2012). The mechanisms for bioactive polysaccharides to exert their function in gut immunity could be described as follows:

(1) To improve the anti-oxidant activity of gut tissue. When the host is suffering from abdominal infection, mounts of free radicals gather in GALT resulting in a rapid decrease of antioxidant activity of gut tissues as well as adverse effects on systemic and mucosal immunologic function. However, lots of dietary bioactive carbohydrates have the ability to improve the anti-oxidant activity in gut tissues, thus improving the host immunity (Chen et al., 2011).

(2) To affect the levels of secretory IgA in intestinal mucosa. Secretory IgA is the primary effector molecule in response to GI mucosal immunity. Many polysaccharides can stimulate the immune function of adaptive system in GALT by strengthening the response of T cells and B cells to antigens, and therefore increase the production of sIgA and eventually enhance the immunity of digestive tract (Chen et al., 2011; Wu et al., 2011).

(3) To prevent the apoptosis of intestinal epithelial cells. The abnormal apoptosis of intestinal epithelial cells accelerate the damage of intestinal mucosa. *Rheum hotaoense* polysaccharides could prevent the apoptosis of ECs by inhibiting the expression of Caspase-3 and the activation of Fas/FasL pathway (Z.-P. Wang et al., 2006).

(4) To regulate the population of M cell. The primary function of M cell is to uptake and present certain antigens to the immune cells in mucosal immune system to induce an effective immune response. Scientific evidence discovered that *Astragalus* polysaccharides could reverse the decrease of M cell population caused by cyclophosphamide injection, thus enhancing the mucosal immunity of piglets (A. Y. Liu et al., 2013).

(5) To modulate the function of macrophage. A co-culture of *Lactobacillus Casei* exopolysaccharide with intestinal macrophages indicated that the exopolysaccharide could improve the phagocytosis of intestinal macrophage and slightly increase the nitric oxide (NO) production, and thus stabilize the homeostasis of gut environment (Sliva et al., 2012; Xu, 2012).

(6) To induce the apoptosis of inflammatory corpuscles. The infiltration of inflammatory cells in gut tissues as well as the consistent activation of these cells can partly account for the disorder of intestinal physiological function. Therefore, it is essential to induce the apoptosis of inflammatory lymphocytes in order to restore the impaired mocosa. After administration of *Portulace oleracea* polysaccharides to experimental ulcerative colitis (UC) rats, the inflammatory corpuscles reduced significantly, accompanied with upregulations in the expressions of Bax and Caspase-3 and a downregulation in Bcl-2 (Sliva et al., 2012).

(7) To modulate the levels of cytokines in intestinal mucosal lymphocytes and peripheral blood (Hur et al., 2012). It is reported that the IBD is accompanied with disturbance of cytokines, such as the increase of proinflammatory factor and the decrease of anti-flammatory factor, or a variation of Th1/Th2 cytokines. However, the consumption of polysaccharides can help regulate these changes, therefore resulting in its therapeutic effect in colitis-associated colorectal disease (Gao, 2010). A pectic-type polysaccharide from peel of *Citrus unshiu* could activate PPs and stimulate the production of hematopoietic growth factors, therefore modulating the intestinal immune system (Suh et al., 2013). Lentinan was able to modulate the inflammatory cytokine mRNA expression in colon tissues of DSS-induced colitis mice (Nishitani et al., 2013).

(8) To modulate the production of chemotactic factor. The administration of *Portulace oleracea* polysaccharides to UC rats showed obvious downregulations in the expressions of chemotactic factor CINC-1 and its receptor CXCR2 mRNA. And these downregulations could reduce the migrating of neutrophile granulocytes to inflammatory sites, thus alleviating the impairing on gut mucosa (T. Zhang et al., 2009).

(9) To promote the production of growth factor. In the inflammation process, the blood viscosity increases, and the blood platelets are activated to release endothelial growth factor (EGF) and platelet-derived growth factor (PDGF). EGF can initiate DNA synthesis, and thus strongly promote the reproduction of ECs; while PDGF can repair the damage in fibroblast and myofibroblast, accelerate the cell proliferation, and, finally, cure the impairing. It is reported that Cuttlebone polysaccharides can help curing the ulcer in intestinal tissues by enhancing the EGF and PDGF levels in mice peripheral blood (Wei et al., 2006).

(10) To promote the production of mucin. The sulfated mucin can resist the enzymolysis of intestinal mucus by pathogenic bacteria, thus protecting the colon mucosa. The level of mucin is said to have a positive correlation with the local inflammation in gut, which means that the lower the mucin level, the more serious the inflammation. Experimental evidence revealed that oral administration of *Portulace oleracea* polysaccharides could protect the intestinal mucosa by revising the decrease of mucoprotein production in UC rats (T. Zhang et al., 2009).

(11) To increase the secretion of NO in microvascular endothelial cells in intestinal mucosa. NO is an important cellular signaling molecule that is involved in a series of physiological and pathological processes. Besides, it is a powerful vasodilator molecule that can improve the vasopermeability. In gut immunity, the effector T cells and B cells are proliferated in micrangiums of intestinal mucosa. Therefore, appropriate modulation of NO level is one way to maintain the microcirculation environment and protect the host nonspecific immunity (L. Li et al., 2003; Liu et al., 2003; Xu, 2012).

Examples of polysaccharides as treatment of IBD and their possible modulating mechanism are summarized in Table 1.

Mechanism of the interaction between polysaccharides and inflammatory bowel disease

IBD is a chronic inflammatory disease developed in GI tract caused by abnormal immune responses, resulting in a series of multifactorial symptoms, including pain in digestive tract, vomiting, diarrhea, rectal bleeding, weight loss, et al. These intestinal disorders include Crohn's disease (CD) and UC (Faubion, 2013; Scott et al., 2013).

The experimental and clinical evidences have indicated that the process of IBD is usually associated with the disruption of gut microbiome and the dysfunction of epithelial barrier and immune cells in gut (Mamula et al., 2012). Dysbiosis of the gut microbiome, including the overgrowth of pathogenic bacteria and a disruption in microbial diversity or loss of functional species, is considered to be one cause for the development of IBD (Manichanh et al., 2012). *E. coli* was observed increasing in both colon and ileum of CD patients. *Myobacterium Avium* subspecies *Paratuberculosis* is said to play a causal role in the pathogenesis of Johne's disease in cattle, a disease similar to human CD, but remains unclear whether it is also one cause in human CD (He et al., 2013). Another consistent finding in numerous researches is the phylum *Firmicutes*, showing a decreased population of *Faecalibacterium prausnitzii* in IBD patients (Faubion, 2013). In addition, the disruption of epithelial barrier may also induce or maintain the inflammation in intestine. In many clinical cases of IBD, barrier dysfunction was directly observed using confocal endomicroscopy. This barrier dysfunction can lead to expansion in intestinal permeability, excessive uptake of antigens, persistent immune stimulation, and, finally, motivating the mucosal inflammation. Another influence brought by barrier dysfunction is the abnormal apoptosis of ECs, especially the death of Paneth cell, which has been recently discovered to induce inflammation in terminal ileal. Lymphocytes in mucosal immunity system are as well of significant importance in the development of IBD, particularly the activated $CD4^+$ T cells. In the process of human IBD, Th17 cells are demonstrated to be involved in Crohn's disease, whereas an atypical Th2 response is observed in ulcerative colitis (Hur et al., 2012).

One of the beneficial strategies for the therapy of IBD is probiotics. Polysaccharides, regarded as probiotic, have been recently applied in the treatment of IBD. The mechanisms for polysaccharides to act as a probiotic in treating with IBD can be described as follows: modulating the composition of gut microbiota to optimistic standard; and regulating the host systemic and mucosal immune responses. Clinical application of konjac glucomannan hydrolysates in the IBD patients showed a therapeutic function, including the improvement in bowel movement and stool consistency, and alleviation of abdominal pain and diarrhea (Suwannaporn et al., 2013). An animal study of *Astragalus* polysaccharides (APS) on TNBS-induced colitis indicated that APS could attenuate the experimental colitis from aspects of downregulating the overactivation of DCs in MLNs and restoring the balance of Treg/Th17 (Dai, 2011). Another experiment on apple polysaccharides revealed that it

Table 1. Effects of polysaccharide on intestinal mucosal immune system.

In vivo experiment				
Polysaccharide	Changes after administration of polysaccharide	Species for the experiments	Model of disease	References
Astragalus polysaccharides	The production of sIgA↑	Kunming mice	Cyclophosphamide-induced mice model	Wu et al. (2011)
	The rato of GATA-3/T-bet ↑ Recover the ratio of Th1/Th2	SD rats	TNBS-induced rats	Gao (2010) Li et al. (2007)
	Modulate the balance of T lymphocyte subsets The levels of sIgA in intestinal mucosa ↑	SD rats	Scalded rats	Huang (2013)
	The expression of MHCII and CD86 in DCs in MLNs ↓ The ratio of Foxp3/RORγ ↑	SD rats	TNBS-induced rats	Dai (2011)
Lentinan	Balance the T lymphocyte subsets	Balb/c mice	DNFB-induced delayed type hypersensitivity mice model	Shen et al. (2007)
	Balance the T lymphocyte subsets	Balb/c mice	Cyclophosphamide-induced immune-suppressed mice model	Zhang (2012)
	Th1 type pro-inflammatory cytokine (IFN-γ and IL-1β) mRNA expression↓	C57BL/6CrSlc mice	DSS-induced colitis mice	Nishitani et al. (2013)
Ganoderma lucidum polysaccharides	The T-SOD activity in intestinal mucosa ↑ The MDA content ↓	Balb/c mice	MTX-induced mucosal injury mice	Chen et al. (2011)
Mushroom *Ganoderma lucidum*	sIgA↑	Balb/c mice	MTX-induced small intestinal damage mice	Chen et al. (2011)
	Reduced infiltration of macrophages in colon tissue	ICR mice	PhIP/DSS induced colon carcinogenesis mice	Sliva et al. (2012)
QiLing polysaccharide	The number of intraepithelial lymphocytes and goblet cells↑ sIgA level ↑	Kunming mice	Cyclophosphamide-induced immune-suppressed mice model	Liao (2010)
Polysaccharide from a two-herb formula (*Lycium barbarum* and *Astragalus membranaceus*)	Serum levels of endotoxin (EDT), diamine oxidase (DAO), and d-lactate (DLA)↓	Sprague-Dawley rats	Acetic acid-induced rat	Zhao et al. (2014)
Pachymaran	Reverse the decrease in the proportion of $CD3^+$ and $CD19^+$ cells in PPs and MLNs	Balb/c mice	Cyclophosphamide-induced mice model	Wang et al. (2011a)
	OVA specific sIgA in mice feces ↑ The expression rate of CD80 and CD86 in PPs ↑	Balb/c mice	Mice immunized with OVA	Wang et al. (2011b)
Rhubarb polysaccharide/ *Rheum tanguticum* polysaccharide	The apoptosis of colonic epithelial cells ↓ The apoptosis of peripheral blood polymorphonuclear neutrophils (PMN) ↑ The expression of Caspase 3, Fas, and FasL protein ↓	Balb/c mice	TNBS-induced UC model	Wang et al. (2006)
	MPO level↓ CD4+ cell in colon↓	Male Sprague-Dawley rats	TNBS-induced colitis rat	Liu et al. (2003)
Schisandrae polysaccharide	sIgA level ↑ The levels of IL-2, IL-4, IFN-γ ↑in the first seven days The number of $CD3^+$, $CD4^+$ cells ↑	Balb/c mice	Healthy mice	Jiang (2013)
Low molecular weight apple polysaccharides	The expression of Cdk-2, cyclin E, and cyclin A↓ The expression of phosphorylated-Cdk-2↑ The levels of p53 and p21 had no significant changes	ICR-mice	AOM-DSS induced colitis-associated colorectal cancer mice	Li, Mei et al. (2012)
A pectic-type polysaccharide from peel of *Citrus unshiu*	Hematopoietic growth factors from Peyer's patch↑	C3H/He mice	Health mice	Suh et al. (2013)

(*Continued on next page*)

Table 1. (Continued).

In vivo experiment				
Polysaccharide	Changes after administration of polysaccharide	Species for the experiments	Model of disease	References
Wheat soluble nonstarch polysaccharides	The height of intestinal villi ↓ The crypt depth of duodenum and iejunum↑	SD rats	Healthy rats	Yonghui et al. (2010)
Lactobacillus Casei exopolysaccharides	Dual-regulate the process of $CD4^+$ T cells to Th17 in PPs Promote the migration of Th17 to PP	Balb/c mice	Healthy rats	Su (2012)
	The proportion of $\gamma\delta$ T lymphocytes ↑ The levels of IL-17 and IFN-γ produced by $\gamma\delta$ T lymphocytes ↑	Balb/c mice	Healthy rats	Bao (2012)

In vitro experiment			
Polysaccharide	Changes after stimulated with polysaccharide	Cell lines	References
Astragalus polysaccharides	Reverse the increase of TNF-α and IL-8 mRNA expression caused by LPS The expression of NF-κB ↓	LPS-damaged IEL-6	Yuan et al. (2008)
Lentinan	Inflammatory cytokine (IL-8) mRNA expression↓ NF-κB translocation into the nucleus↓ Surface TNFR1 signaling↓	Caco-2 cells	Nishitani et al. (2013)
Rhubarb polysaccharide	The restitution, proliferation, and differentiation of the cells ↑	IEL-6 cells	Liu (2005)
	The apoptotic ratio ↓ The activation of Caspase 3 ↓ The expression of anti-apoptotic protein Bcl-2 ↑	H_2O_2-induced insult in IEL-6 cells	
Modified apple polysccharides	Suppressed the nuclear translocation of NF-κB p65 The expression of TLR4, COX-2, MMP9, MMP2, iNOS, and PGE2 ↓ The protein expression of the inhibitor of $\kappa B\alpha$ and NF-κB p65 in cytoplasm↑	LPS-damaged HT-29 and SW620	Zhang et al. (2013)
Low molecular weight apple polysaccharides	A dose-dependent inhibition of the progression from G0/G1 to the S phase in HT-29 cell lines The expression of Cdk-2, cyclin E, and cyclin A↓ The expression of phosphorylated-Cdk-2↑ The levels of p53 and p21 had no significant changes	HT-29	Li, Liu et al. (2012), Li, Mei et al. (2012)
Bean polysaccharide	Apoptosis genes, SIAH1, PRKCA, and negative regulation of the cell cycle gene MSH2↑↑(they were the highest up-regulated genes with 30.5-, 18.4-, and 9.8-fold, respectively)	HT-29	Campos-Vega et al. (2010)
Lactobacillus Casei exopolysaccharides	The phagocytosis of intestinal macrophages ↑ The production of NO ↑	Intestinal macrophages from Balb/c mice	Xu (2012)
	The proliferation of T lymphocytes ↑ The levels of IL-17 secreted by T lymphocytes in IEL, LPL, PPL, MLNL↑ The levels of TNF-α T lymphocytes in MLNL ↑ The levels of TNF-α T lymphocytes in IEL ↓	T lymphocytes derived from mice intestine	Bao (2012)
	The expressions of IA/IE and co-stimulatory factor CD80 in DCs derived from mice intestine ↑ Promote the migration of DCs from blood to intestine	Dendritic cells derived from mice intestine	Yang (2012)

can prevent further canceration of inflammation in intestine by inhibiting the activation of TLR4/MyD88/NF-κB pathway (Zhang et al., 2013).

Perspective

The human gut harbors a huge number of diversity bacteria, which play a key role in keeping us healthy. The gut microbiota can be mainly affected by host genes, diet, age, and environment. Maternal exposure to environmental stimuli during pregnancy and infant's prenatal exposure to bacteria both have great influences on the development of body's immune system. Additionally, many of the disease processes are also reported to be relevant to the dysbiosis of gut microbiota. Gut-associated lymphoid tissue has a large collection of immune cells, including T lymphocytes, B lymphocytes, macrophages, and DCs. Thus, numerous immune responses take place in gut, and these immune responses are closely connected with the host systemic immunity. Based on these reasons, it is meaningful to keep the balance of intestinal microbial ecosystem and benefit the GALT.

Dietary carbohydrate, existing in a wide range of natural sources, recently has been proved to benefit the gut immunity from both aspects of keeping the homeostasis of gut microbial ecosystem and stimulating the development of intestinal mucosal immune system. For instance, the nondigestive polysaccharides can be further fermented into SCFA by gut microbiota, thus providing energy for host and bacteria. In addition, the dietary polysaccharides can also affect the proliferation of intestinal epithelial cells and the activation of intestinal immune cells. Therefore, it is not surprising that dietary polysaccharides have the therapeutic ability in the treatment of IBD.

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