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Prebiotics, probiotics, synbiotics, and the immune system: experimental data and clinical evidence

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Purpose of review
The intestinal immune system is constantly exposed to foreign antigens, which for the most part should be tolerated. Certain probiotics, prebiotics, and synbiotics are able to influence immune responses. In this review, we highlight the recent publications (within the last 2 years) that have substantially progressed this field.

Recent findings
The immunological mechanisms underpinning probiotics, prebiotics, and synbiotics effects continue to be better defined with novel mechanisms being described for dendritic cells, epithelial cells, T regulatory cells, effector lymphocytes, natural killer T cells, and B cells. Many of the mechanisms being described are bacterial strain or metabolite specific, and should not be extrapolated to other probiotics or prebiotics. In addition, the timing of intervention seems to be important, with potentially the greatest effects being observed early in life.

Summary
In this review, we discuss the recent findings relating to probiotics, prebiotics, and synbiotics, specifically their effects on immunological functions.

Keywords
adaptive immune system, innate immune system, prebiotics, probiotics, synbiotics

INTRODUCTION

The mammalian gastrointestinal tract is a highly evolved system specialized to perform the essential functions of nutrient digestion, absorption, and waste disposal. The intestinal immune system has the unenviable task of maintaining intestinal integrity in the presence of vast quantities of external or foreign antigens. Sophisticated cellular and molecular networks need to be constantly coordinated in order to tolerate nonpathogenic antigens, while also protective immune responses to potential pathogens must be maintained and can be induced effectively on demand. Inappropriate immune response to bacterial or dietary antigens is a significant component in several intestinal diseases, including inflammatory bowel disease, irritable bowel syndrome, and food allergies [1,2].

The balance between immune tolerance and inflammation is regulated in part by the crosstalk between innate and adaptive immune cells and the intestinal microbiota. Disrupted communication between the microbiome and the host because of altered composition and metabolism is thought to negatively influence the intestinal immune homeostatic networks. The deliberate modification of microbial species and their metabolism has led to the probiotic and prebiotic concepts [3].

Probiotics can be defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. Notably, the definition of a probiotic does not differentiate between the wide range of potential health benefits and it is clear that not all probiotics will influence the immune system in the same way. In this review, we have focused on the probiotic studies with immunological endpoints, but these findings cannot be extrapolated to other probiotic strains. In addition, we have included microbial species not currently used in the industry as probiotics, even

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KEY POINTS

- Specific probiotics, prebiotics, or their combination significantly influence host immunological networks.
- The immune responses to microbial components (e.g. polysaccharides) and their metabolites (e.g. SCFA) are being better characterized at a molecular level.
- The immune-modulatory effects of probiotics, prebiotics, or synbiotics may be most potent when administered early in life.

though these microbes fulfill the definition provided above.

Prebiotics can be defined as selectively fermented ingredients that allow specific changes, both in the composition and in the activity of the gastrointestinal microflora, which confer benefits upon host well being and health. Prebiotics typically are fibers that cannot be digested by the host, but are metabolized by the colonic microbiome resulting in expansion of certain bacterial species and the release of metabolites, such as short-chain fatty acids (SCFAs). As with the probiotic definition, not all prebiotics will have the same effect on immunological functions.

The combination of probiotics and prebiotics is termed 'synbiotics'.

INNATE IMMUNE SYSTEM

The innate immune system is comprised of many different cell types, and these cells are often the first cells to come in contact with intestinal microbes and their metabolic products. The most commonly described cell types in the probiotics and prebiotics literature are dendritic cells and epithelial cells.

Dendritic cells

Intestinal dendritic cells are located within specific intestinal lymphoid tissues, collectively termed gut-associated lymphoid tissues (GALT), or diffusely distributed throughout the intestinal lamina propria [4]. Dendritic cells are the primary cell type involved as 'sensors' of microbial ligands through activation of innate immune receptors (e.g. Toll-like receptors and c-type lectin receptors). The signaling pathways triggered by bacterial-derived molecules allow for changes in dendritic cell phenotypes and cytokine secretion, which underlie the integration of microbial and host metabolism with immune functions. Metabolism of vitamin A to retinoic acid is a key immunomodulatory activity associated with intestinal dendritic cells [5▪]. Certain, but not all, probiotic microbes can induce retinoic acid metabolism by human dendritic cells *in vitro* and by murine $CD103^+$ dendritic cells within the small intestine lamina propria [6▪,7]. In addition to vitamin A metabolism, induction of another dendritic cell metabolic enzyme, heme oxygenase-1 (HO-1), was shown to be required for the induction of mucosal T regulatory (T_{REG}) cells within the mesenteric lymph nodes by *Lactobacillus rhamnosus* [8].

Bacterial cell wall components and metabolites have been associated with the immunoregulatory effects on dendritic cells. For example, major histocompatibility complex (MHC)-II-dependent presentation of segmented filamentous bacteria antigens by intestinal $CD11c^+$ dendritic cells is crucial for the local induction of T_H17 lymphocytes [9▪▪]. In addition, capsular polysaccharide A (PSA) from *Bacteroides fragilis* has been shown to interact directly with mouse plasmacytoid dendritic cells via TLR-2. PSA-exposed plasmacytoid dendritic cells express molecules involved in protection against colitis and stimulated $CD4^+$ cells to secrete IL-10 [10▪]. An exopolysaccharide from *Bacillus subtilis* prevents gut inflammation stimulated by *Citrobacter rodentium*, which is dependent on TLR-4 and MyD88 signaling [11▪].

The production of SCFAs occurs in the colon following fermentation of dietary fibers, such as prebiotics [12]. Abnormalities in the production of these metabolites (because of dietary factors and dysbiosis) might play a role in the pathogenesis of type 2 diabetes, obesity, inflammatory bowel disease, colorectal cancer, and allergies [13]. Among the SCFAs, butyrate seems to be more potent than acetate or propionate in inducing immunomodulatory effects. Butyrate influences the activity of histone deacetylases (HDAC), which is responsible for decreasing dendritic cell IL-12 and IL-6 cytokine secretion and allows dendritic cells to promote T_{REG} cells. Propionate can also contribute to the induction of Foxp3 expression by dendritic cells, whereas acetate does not have this activity possibly because of the lack of HDAC activity [14▪]. Butyrate also inhibits intestinal macrophage HDAC [15▪▪]. Another recent study has confirmed and extended the observation that butyrate promotes dendritic cell regulatory activity, resulting in the induction of T_{REG} cells and IL-10-secreting T cells. These effects were mediated by the G-protein-coupled receptor Gpr109a on colonic dendritic cells and macrophages [16▪]. In contrast, butyrate has also been shown to promote IL-23 secretion by murine dendritic cells, which may promote T_H17 responses under certain circumstances [17].

Histamine is another important mucosal metabolite secreted by the gut microbes, and mucosal histamine levels are increased in patients with irritable bowel syndrome and inflammatory bowel disease [18▪]. Histamine is able to decrease chemokine and proinflammatory cytokine secretion induced by the Toll-like-receptor-stimulated dendritic cells, while increasing IL-10 production [19▪]. Histamine exerted this effect by activating the histamine 2 receptor (H_2R) on dendritic cells and the signaling mechanism required cyclic adenosine monophosphate (cAMP) and exchange protein directly activated by cAMP (EPAC). Administration of a histamine-secreting *Lactobacillus* strain to mice resulted in rapid weight loss and enhanced Peyer's patch cytokine secretion, which was exaggerated in H_2R-deficient animals [20▪▪].

Epithelial cells

Epithelial cells play an essential role in nutrient absorption. Pathogen-induced reductions in epithelial cell digestive enzyme activity can be blocked by *Bifidobacterium infantis* 35624, possibly via modulation of mucosal inflammatory responses [21,22▪]. In addition to their absorptive function, epithelial cells form a mucosal barrier that protects host tissue from damaging agents such as luminal pathogens and toxic products. One protective barrier mechanism is the production and secretion of antimicrobial peptides, such as defensins and cathelicidins. Probiotic strains have been shown to differentially regulate defensin expression and protein secretion, which is influenced by local inflammatory mediators [23]. Autophagy is an important adaptive response to stress, which promotes cell survival and is required for the maintenance of the epithelial barrier. A number of Bifidobacteria have been recently described that promote autophagy in an intestinal cell line [24]. The mucus layer coating the gastrointestinal tract is an important barrier component and probiotics have been shown to promote mucin production by goblet cells in the intestine. Recently, p40 from *Lactobacillus* GG was demonstrated to be sufficient for the stimulation of mucin production through transactivation of the epidermal growth factor receptor [25▪]. Excessive epithelial cell responses to microbial ligands result in local inflammatory responses, which disrupt the epithelial barrier. A wide range of probiotic microbes have been demonstrated to suppress epithelial cell proinflammatory chemokine responses [26▪,27,28]. However, not all chemokine responses are impacted to the same extent by every probiotic strain and a single probiotic strain may reduce the expression of certain chemokines, while increasing the expression of others. For example, *Bifidobacterium bifidum* PRL2010 suppresses CCL22 expression but enhances CCL19 expression, suggesting that strain-specific and chemokine-specific responses are induced by probiotics [29▪]. In addition, prebiotics themselves, or SCFAs, can also modulate epithelial barrier function, production of antimicrobial peptides, and secretion of proinflammatory mediators [30,31].

ADAPTIVE IMMUNE SYSTEM

The adaptive immune system receives polarizing signals from the innate cells to expand an appropriately controlled lymphocyte response to bacterial and metabolic factors. Recent evidence has suggested a role for probiotic and prebiotic effects on T_{REG} cells, effector T cells, natural killer T (NKT) cells, and B cells.

T lymphocytes

The beneficial effect of prebiotics, probiotics, and synbiotics against diseases such as allergy or colitis is often associated with enhancement of T_{REG} cells [32▪,33]. Specific probiotics, but not all, have been shown to induce an increase in T_{REG} cells. Notably, consumption of *B. infantis* 35624 by healthy human volunteers resulted in an increased proportion of $Foxp3^+$ lymphocytes in peripheral blood, whereas administration of this probiotic to psoriasis patients, chronic fatigue syndrome patients, or ulcerative colitis patients consistently resulted in reduced levels of serum proinflammatory biomarkers such as C-reactive protein, possibly mediated by increased numbers of T_{REG} cells [7,34▪▪].

In addition to probiotics, effects on lymphocytes can also be mediated by SCFAs such as acetate, propionate, butyrate, and *n*-butyrate. Oral administration of a mixture of 17 Clostridia strains to mice attenuated the severity of colitis and allergic diarrhea in a T_{REG}–TGF-β-dependent mechanism. This process is most likely because of SCFAs produced by the Clostridia strains [35,36▪▪]. G-protein-coupled receptor (GPCR) 43 has been described as receptor for SCFAs. GPR43 signaling ameliorates diseases such as colitis, inflammatory arthritis, and allergic airway diseases [35,37▪]. In addition to the expression on neutrophils and eosinophils, GPCR43 is expressed on colonic inducible T_{REG} cells and promotes their expansion and IL-10 secretion.

As already described above for dendritic cells, SCFAs inhibit HDAC activity also in lymphocytes enhancing histone H3 acetylation in the promoter and conserved noncoding sequence regions of the *Foxp3* locus [35,38▪].

Probiotic and commensal bacteria have been shown to suppress T_H17 responses through both direct and indirect mechanisms. T_H17 cells secrete IL-17 that induces tissue inflammation. Probiotic strains inhibit T_H17 and IL-17 activity by inducing T_{REG} and T_H1 subsets, by induction of IL-27 production, which suppress the generation of IL-17 and induce IL-10 or by stimulation of TLR9 on T_H17 cells [39].

Natural killer T cells

NKT cells are central mediators of intestinal inflammation and pathogenic NKT cell activation is mediated by $CD1d^+$ bone-marrow-derived cells, whereas $CD1d^+$ epithelial cells protect against intestinal inflammation [40]. It has been shown that NKT cells can be influenced by the gut microflora and certain probiotic lipid antigens may directly activate NKT cells [41,42]. Recently, a healthy human volunteer study showed that the combination of xylo-oligosaccharide with *Bifidobacterium animalis* reduced CD16/CD56 expression on NKT cells and reduced IL-10 secretion from peripheral blood mononuclear cells in response to lipopolysaccharide [43▪]. The functional consequences of altered NKT cell activation by the microbiome in humans remain to be determined.

B cells

B lymphocytes have an essential role in humoral immune responses via their secretion of antigen-specific antibodies. In addition, B cells can limit aggressive immune reactivity. B cells regulate immune responses mainly via IL-10, which has been shown in the experimental models of infection, allergic inflammation, and tolerance [44].

B-cell-dependent modulation of the microbiome was shown in IgA-deficient mice. IgA-deficient mice had persistent intestinal colonization with γ-Proteobacteria that cause sustained intestinal inflammation and increased susceptibility to neonatal and adult models of intestinal injury. The group also identified an IgA-dependent mechanism responsible for the maturation of the intestinal microbiota in mice [45]. Recently, another group showed that the number of gut-homing IgG^+ and IgA^+ B cells was significantly higher in infants compared with adults. This suggests that activation of naïve B cells in the gut overlaps with the establishment of the gut microbiota in humans [46]. Oral administration of *Lactobacillus gasseri* SBT2055 (LG2055) induced IgA production and increased the number of IgA^+ cells in Peyer's patches and in the lamina propria. Combined stimulation of B cells with B-cell activating factor and LG2055 enhanced the induction of IgA production. IgA plays an important role in host defense against mucosally transmitted pathogens, prevents commensal bacteria from binding to epithelial cells, and neutralizes their toxins to maintain homeostasis at the mucosal surfaces [47].

It was recently shown that the gut microbiota induces dendritic cells and macrophages to produce IL-1β and IL-6, which both drive T_H17 differentiation and arthritis. The same signals also induced the differentiation of IL-10-producing B regulatory cells. These data suggest that the commensal microbiota is important for inducing both proinflammatory and regulatory responses in order to rapidly clear infections and minimize the inflammation-associated tissue damage [48▪▪]. Interestingly, supplementation with xylo-oligosaccharide and *B. animalis* in a human study led to reduced expression of CD19 on B cells [43▪].

AGE-DEPENDENT EFFECTS OF PROBIOTICS, PREBIOTICS, AND SYNBIOTICS

The timing of bacterial colonization early in life is thought to be important for appropriate immune education and the transmission from mother to the fetus during pregnancy and birth is being better described. Cultures of meconium have shown diverse groups of Gram-positive and Gram-negative bacteria, possibly not all derived after delivery. The development of the gut microbiome is a dynamic process and early colonization with Bacteroides and *Bifidobacterium* species might play a crucial role in the development of immune regulation [49▪]. Factors that can influence early-life colonization include antibiotic treatment, method of delivery, maternal and infant diet, and biodiversity in the home, surrounding environment and in family members.

The immune system at birth is dominated by the T_H2 cells. However, the human fetus has a functional immune system at a relative early status of development comprising not only $CD4^+$ and $CD8^+$ T cells, but also $FOXP3^+$ T_{REG} cells. One concept gaining support is that the developing fetus may become educated by whole bacteria or their genetic material that is provided via maternal serum. DNA from Bifidobacteria and Lactobacilli, two genera typically used as probiotics, are found in human placenta. In contrast, in-utero exposure to potentially pathogenic bacteria such as *Ureaplasma* species leads to immune dysregulation, commonly ending in fatal complications [50]. Maternal consumption

of probiotic-containing food components may reduce the risk for childhood allergic diseases, and mouse models demonstrate a reduced risk of inflammatory bowel diseases [50]. Epigenetic mechanisms may be critical as application of *Acinetobacter lwoffii* to pregnant mice reduced the airway hypersensitivity response of the offspring. The promoter region of IFN-γ in $CD4^+$ T cells of the offspring had high levels of histone-4 acetylation, associated with enhanced transcription, whereas the IL-4 promoter region had lower levels of histone-4 acetylation. Moreover, exposure of pregnant mothers to the farm environment, which has high levels of *A. lwoffii*, was associated with DNA demethylation of the *Foxp3* locus and methylation of the T_H2-associated genes RAD50 and IL-13 [50].

As the gut microbiota composition during the first months of life seems to be important for the development of appropriate immune regulatory networks and thereby influences later life disease risk, intervention with probiotics, prebiotics, or synbiotics might be most effective at this age or even during pregnancy.

CONCLUSION

Appropriately selected probiotics, prebiotics, or their combination exert potent effects on the immune system. Of particular interest are the recent findings on the molecular mechanisms underpinning the effects of SCFAs on immune cells. However, it is highly likely that SCFAs are only one example of the many bacterial metabolites which influence immune cells. A better description of the bacterial strains and metabolites which influence immune function will allow for improved design of future probiotic and prebiotic cocktails for the prevention and treatment of immunological disorders.

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Conflicts of interest

L.O.M. is a consultant to Alimentary Health Ltd. and has received research funding from GSK. R.F. and M.A. have no conflicts of interest.

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