

# Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions

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## ABSTRACT

Probiotics and prebiotics, alone or together (synbiotics), can influence the intestinal microbiota and modulate the immune response. They may therefore be tools that can prevent or alleviate certain pathologies involving the gut immune system, such as allergies for which no treatment is yet available. This review focuses first on the definitions of probiotics, prebiotics, and synbiotics and key cells in the gut immune system. It then discusses their effects on mucosal immune stimulation. Experimental findings suggest that different probiotic species have similar effects on innate immunity by improving the mechanisms of pathogen destruction. On the contrary, their impacts seem to be variable on the adaptive immune system. Prebiotics can also exert an influence on the gut immune system via the stimulation of the autochthonous bacteria metabolism. Finally, this review focuses on the effects of food supplements on allergy. Different studies performed in humans or rodents have supported a potential role for selected probiotics and prebiotics in reducing some allergic parameters. Probiotic effects on allergy treatment are unclear, especially in human studies. However, they are potentially effective at short-term for prevention when they are administered in perinatal conditions. A clinical study performed with an infant cohort revealed a beneficial effect of prebiotics in preventing allergic manifestations at long-term. Further studies are nonetheless essential to confirm these findings. Food supplements offer potential tools for the prevention or treatment of allergy, but insufficient evidence is available at present to recommend their use in clinical practice. *J. Leukoc. Biol.* 89: 685–695; 2011.

## Introduction

In 1998, probiotics were described as: “live microorganisms which, when consumed in adequate amounts, confer a health

benefit on the host” [1]. Prebiotics have been defined as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or the activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health” [2, 3]. A synbiotic is defined as a product that contains both prebiotic(s) and probiotic(s). These food supplements have numerous and complex effects on the intestinal microbiota and gut immune system. Their properties mean that they may have some interesting applications in some pathological situations involving the gut immune system [4–6]. However, at present, there is no evidence (or only some preliminary evidence) of their potential therapeutic application in the prevention or treatment of gut pathologies such as inflammatory bowel diseases [7] or allergies [8, 9]. Epidemiological studies have indicated that the prevalence of allergic disorders, such as allergic rhinitis, asthma, and eczema, has risen during recent decades in many Western countries [10, 11]. This increased disease incidence seems to be associated with a variety of environmental factors: the hygiene hypothesis, antibiotic use, increased chemical exposure, lifestyle changes, or a Western-style diet low in fiber and antioxidants [12–14]. As yet, there is no treatment that can cure allergies, thus enhancing the interest of using functional foods enriched in prebiotics or probiotics. This review provides an overview of data about probiotics, prebiotics, and synbiotics and discusses the varying definitions of them suggested by different investigators. Key cells of the gut immune system are described, and the effects of these food supplements on mucosal immune stimulation, and ultimately, allergic diseases are considered. Our aim has been to determine whether there are sufficient, valid arguments to support the use of these food supplements in the prevention and treatment of allergic diseases.

## DEFINITIONS OF PROBIOTICS, PREBIOTICS, AND SYNBIOTICS

### Probiotics

Probiotics are microorganisms (bacteria, yeast) that exert a beneficial effect on host health [15]. When some of these mi-

Abbreviations: AD=atopic dermatitis, DTH=delayed-type hypersensitivity, FOS=fructooligosaccharides, GOS=galactooligosaccharides, LGG=*Lactobacillus rhamnosus* GG, NOD=nucleotide-binding oligomerization domain, PP=Peyer's patches, PRR=pattern recognition receptors, SCFA=short-chain fatty acid, SOS=soybean oligosaccharides, Treg=T regulatory cell

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croorganisms are ingested, they are able to resist the physico-chemical conditions prevailing in the digestive tract [16], and the strains most frequently used as probiotics belong to the genera *Bifidobacterium* and *Lactobacillus* [17, 18]. Allochthonous lactobacilli and bifidobacteria (probiotics) ingested as food or supplements need to be differentiated from the autochthonous organisms that constitute human commensal microbiota. The populations of these bacterial genera, whatever their origin (allochthonous or autochthonous), differ between individuals and can be affected by environmental factors such as drugs and diet. Probiotics can act on the gut immune system (as discussed later). They also play non-immune, protective roles by directly blocking intestinal pathogenic microbes and enhancing mucosal integrity via epithelial cell stimulation.

#### Directly blocking intestinal pathogenic microbes

Probiotics can directly inhibit the growth of pathogens by producing antibacterial substances, including bacteriocins (such as lantibiotics) and different acids (acetic, lactic, and propionic acid) [19, 20]. They can also decrease the adhesion of pathogens and their toxins to the gastrointestinal tract through their ability to adhere to intestinal epithelial cells. In vitro studies have demonstrated that probiotics possess lectin-like adhesion components able to bind on carbohydrates from glycoconjugate receptors of the epithelial cell surface [21–23], thus blocking pathogen binding to intestinal epithelial cells. Moreover, some probiotic strains belonging to the *Lactobacillus* genus have displayed in vitro abilities to bind to the surface of enterocytes [24].

#### Enhancing mucosal integrity

First, probiotic bacteria can enhance the integrity of the intestinal barrier. Indeed, Ewaschuck et al. [25] established that *Bifidobacterium infantis* increases resistance in an in vitro model of the intestinal barrier (T84 human epithelial cells), and other probiotics, such as *Lactobacillus plantarum*, act on tight junctions by increasing the expression of *zonula occludens* proteins and occludins [26]. Probiotics are also able to induce the production of cytoprotective substances by enterocytes, such as the heat shock proteins [27] and antimicrobial peptides, e.g., defensin [28] and mucin [29, 30], to prevent bacterial adherence. Finally, they may be involved in preventing cytokine-induced epithelial damage by promoting the survival of intestinal epithelial cells by activating antiapoptotic and inhibiting proapoptotic pathways [31].

In summary, probiotics are able to exert an impact on the intestinal environment through different key non-immune mechanisms.

#### Prebiotics

A variety of molecules can be prebiotics, but the great majority is dietary fibers, such as oligosaccharides. Their principal effect is related to the metabolism of the microbiota. Indeed, if no dietary fiber is present in the colon, anaerobic bacteria draw their energy from protein fermentation. This metabolism leads to the production of toxic and potentially carcinogenic compounds (such as ammoniac or phenolic compounds) [32, 33]. By contrast, the fermentation of carbohydrate (such as dietary fiber) generates SCFAs, such as acetate, propionate, or butyrate [34], which are not toxic to the host [32], and consti-

tutes potential fuel for epithelial cells. For instance, in an in vivo rat model, a fructan (inulin, FOS)-enriched diet has increased the production of SCFA [35]. The interesting properties of SCFAs on the intestinal barrier and gut immune system are discussed below. Several oligosaccharides are considered to be prebiotics, such as inulin, GOS, FOS, SOS, and xylooligosaccharides. The most widely studied have been inulin, GOS, FOS, and a mixture of GOS and inulin [36, 37]. These molecules are resistant to in vitro digestion by pancreatic and brush-border enzymes [38]. Chicory is the principal industrial source of inulin, from which FOS are often obtained by partial enzymatic hydrolysis [39]. GOS are produced from lactose using  $\beta$ -galactosidase [40]. Each bacterial genus or species has a preferential substrate. Most strains belonging to the *Bifidobacterium* and *Lactobacillus* genera preferentially use fructans rather than glucose as a substrate [41, 42]. However, other bacteria such as Clostridia and Bacteroides are also able to grow on fructans [35, 43]. Nevertheless, their growth is less efficient than that of *Bifidobacterium* because of less-efficient substrate absorption (notably on oligofructose) [43]. Thus, prebiotics act first to ensure selective stimulation of the activity of beneficial autochthonous bacterial strains [38], but they do not stimulate the same genera of intestinal microbiota in humans and rodents. Indeed, in humans, prebiotics specifically increase bifidobacterial populations in fecal samples [33, 44, 45], whereas lactobacilli populations are increased significantly in the fecal microbiota of rodents (rat, mouse) [46, 47]. Last but not least, some prebiotics (GOS) can exert a direct antimicrobial effect, as they can adhere to the binding sites of bacteria on the enterocyte surface and thus, block the adhesion of pathogenic bacteria to intestinal epithelial cells [42, 48].

In conclusion, although it is clear that prebiotics have some effects on microbiota (modification, stimulation, antipathogenic effect), little is known about the specific action of each type of oligosaccharide on the various genera and species that make up the microbiota.

#### Synbiotics

A combination of probiotic(s) and prebiotic(s) constitutes a synbiotic [2, 16], which can stimulate and increase the survival of probiotic and autochthonous-specific strains in the intestinal tract. Su et al. [49] administered some combinations to mice, each containing one prebiotic (FOS, SOS, or inulin) and one probiotic (*Lactobacillus acidophilus*, *Bifidobacterium lactis*, or *Lactobacillus casei*). They then analyzed the amounts and survival of each probiotic strain in fecal samples. A SOS- or FOS-containing diet appeared to stimulate the growth of *L. acidophilus* and sustain its highest level. FOS and inulin dietary treatment exerted the same effects on *B. lactis*. SOS, FOS, or inulin improved the survival and retention time of *L. casei*. Such effects have also been demonstrated in humans, as the administration of probiotic LGG with GOS was seen to significantly increase the amount of allochthonous *Lactobacillus rhamnosus* and autochthonous bifidobacteria and lactobacilli in feces from treated children [50].

Human breast milk contains oligosaccharides (more diverse and more complex than those found in many animal milks) [51] and lactic acid bacteria, which may be potential probiot-

ics [52, 53]. Breast milk can therefore be considered as a synbiotic food. It has been evidenced that bacteria from human milk can colonize the gut of a breast-fed child [53]. Some studies have also suggested that oligosaccharides from breast milk may influence the establishment of autochthonous microbiota in breast-fed children [17]. Indeed, differences in terms of microbiota composition have been observed between breast-fed and formula-fed children. In particular, it has been established that the *Bifidobacterium* genus is more common in the feces of breast-fed infants [17, 51].

## KEY CELLS OF THE GUT IMMUNE SYSTEM AND THEIR INTERACTIONS WITH MICROORGANISMS

### Cells for antigen absorption

The induction sites of the immune response in the gut correspond to follicles of GALT organized at the level of PP. M cells present in PP play a key role because of their ability to transport macromolecules, microorganisms, and inert particles from the lumen into lymphoid tissue through adsorptive endocytosis. The uptake of antigens, macromolecules, and microorganisms can also occur through active transepithelial vesicular transport in enterocytes [54] and M cells [55]. Finally, DCs may extend their dendrites through epithelial tight junctions and thus, capture luminal antigens directly [56]. When antigenic molecules have been transported across the intestinal barrier, they can then stimulate the innate and adaptive immune systems.

### Cells of innate immunity

These cells act as the first line of defense against pathogens but are not overly specific in their ability to recognize their targets. Key players in the innate immune response include phagocytic cells, such as neutrophils, monocytes, macrophages, and NK cells.

### Cells of adaptive immunity

DCs, macrophages, and monocytes provide an interface between the innate and adaptive immune systems, as they act as professional APCs. This "bridging" role is crucial to initiating the adaptive immune response, as T cells do not respond to free antigens but only to those presented by APCs. In adaptive immunity, distinct subsets of  $CD4^+$  Th cells (Th1, Th2, or Th17) are described on the basis of their cytokine secretion profiles after the activation and differentiation of naive  $CD4^+$  cells [57] (see Fig. 2).

#### IL-12

IL-12 enables the differentiation of naive T cells into Th1 lymphocytes, which are involved in the tolerance and defense mechanisms of the host against pathogens via the production of different inflammatory cytokines such as IL-2 and IFN- $\gamma$  [58].

#### IL-4

IL-4 enables the differentiation of naive T cells into Th2 lymphocytes. Th2 cells produce cytokines (IL-4, IL-5) that can activate B lymphocytes so that they produce IgE. They are impli-

cated in the mechanisms of parasite defense, atopy, and allergic reaction [59].

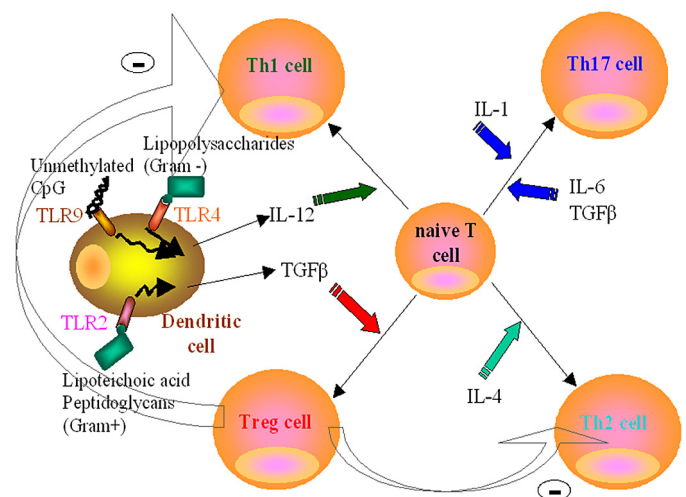
#### TGF- $\beta$ , IL-6, and IL-1

TGF- $\beta$ , IL-6, and IL-1 are involved in the generation of Th17 cells. These cells secrete IL-17 and IL-22, which are critical to regulating tissue inflammation [60]. Th17 cells have been shown to protect against extracellular bacteria and fungal infections, as well as contributing to some autoimmune responses [61].

Naïve  $CD4^+$  T cells are also converted into potent immunosuppressive cells,  $CD4^+CD25^+$  Tregs, by TGF- $\beta$  [62]. Tregs, through their IL-10 and TGF- $\beta$  production, are able to check the immune response, block Th1 (autoimmune diseases) or Th2 (allergic diseases) hyperpolarization, and thus, regulate the Th1/Th2 balance.

## The recognition of microorganisms and their role in immune response induction

DCs recognize different pathogens via microbial PRRs and translate these signals into factors that polarize Th1 and Th2 cells [61, 63]. TLRs, which discriminate between different types of pathogens, are the most common PRRs (Fig. 1). The binding of TLR4 or TLR9 to bacterial ligands, such as unmethylated CpG and LPS, turns immature DCs into IL-12-producing and Th1-promoting DCs [64, 65]. The PRR known to mediate the differentiation of Tregs and Th17 cells appears to be TLR2, which is able to recognize lipoteichoic acid on Gram $^+$  bacteria [65, 66]. The PRRs that mediate Th2-promoting DCs still need to be determined. NOD proteins are homologous to TLR, as they contain leucine-rich repeats and recognize pathogenic components at the cell surface. NOD2 is expressed by intestinal epithelial cells, can fix LPS and peptidoglycans, and activates LPS-mediated pathways such as NF- $\kappa$ B [67]. Consequently, NOD2 serves as a key component in the innate mucosal response to luminal bacteria.



**Figure 1. Mechanisms underlying the differentiation of naive  $CD4^+$  T cells into  $CD4^+$  T cell subsets.** Th1, Th2, and Th17 cells and Tregs. Influence of cytokine production by DCs and the triggering of TLR by pathogenic molecules.



TLR and NOD proteins can be activated by specific probiotic strains. Indeed, Tobita et al. [68] established that heat-treated *Lactobacillus crispatus* KT strains could induce TLR2 and NOD2 activation in C3H/HeN mouse PP.

Numerous actors in the gut immune system have now been presented. So what are the effects of food supplements, such as probiotics or prebiotics, on these immune actors?

## EFFECTS OF PROBIOTICS AND PREBIOTICS ON THE GUT IMMUNE SYSTEM

### Immunomodulatory properties of probiotics

Probiotic antigenic fragments (e.g., cell wall compounds) are able to cross the intestinal barrier via intestinal epithelial cells [69] and M cells [70] at the level of PP. They are then processed and presented to the immune system and modulate the innate and adaptive response (Fig. 2).

#### Modulation of innate immunity

Probiotics are endowed with antiviral properties, in that they increase the cytotoxic potential of NK cells and phagocytosis capacity of macrophages [71]. Indeed, cell wall components, such as the lipoteichoic acid of Gram<sup>+</sup> bacteria (bifidobacteria or lactobacilli), can stimulate NO synthase [72] and induce the production of NO (a virus-infected cell death mechanism) by macrophages (via TNF- $\alpha$  secretion). This mechanism involves an up-regulation of important surface phagocytosis receptors such as Fc $\gamma$ RIII [73] and TLR [74].

#### Modulation of the adaptive immune response

Many probiotic strains seem to be capable of stimulating the production of IgAs by B cells, which bind antigens and thereby, limit their access to the epithelium [71]. In children with viral gastroenteritis caused by rotavirus, probiotics such as LGG have been shown to stimulate rotavirus-specific IgA anti-

body responses, which are theoretically significant in the prevention of reinfections. They can thus reduce the number and duration of episodes of diarrhea [75].

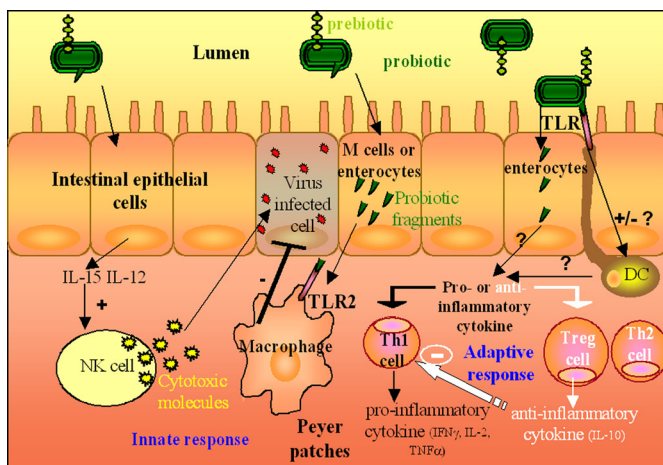
Probiotics interact with a wide variety of cells (enterocytes, DC, Th1, Th2, Treg) in the intestine and may modulate the immune response toward a pro- or anti-inflammatory action. This appears to be a strongly strain-dependent effect.

First, it has been shown in in vitro cell models (enterocyte models, such as HT-29, caco-2, and DC derived from PBMC) that probiotics can influence the production of cytokines by intestinal APC and, initiate the orientation of the adaptive response. Indeed, it has been demonstrated that *Lactobacillus sakei* induces the expression of IL-1 $\beta$ , IL-8, and TNF- $\alpha$  (proinflammatory), whereas *Lactobacillus johnsonii* stimulates the production of TGF- $\beta$  (anti-inflammatory) in Caco-2 cells [76].

Second, it has been observed in animal models that probiotic strains can modulate the Th1/Th2 balance toward Th1 activation (proinflammatory effect) or Th1 inhibition (anti-inflammatory effect). Using a food allergy model to study the Th2 response in mice, Shida et al. [77] showed that an i.p. injection of heat-killed *L. casei* Shirota induced a rise in serum IL-12 levels and a skewing of the cytokine profile from Th2 to Th1 (proinflammatory effect). Vidal et al. [78] reported that supplementation with *Lactobacillus paracasei* also enhanced Th1 cell-dependent immune responses in a mouse model. However, recent studies have supported the potential anti-inflammatory effect of *Lactobacillus* strains in rat colitis [79, 80]. Probiotics may also favor the production of an anti-inflammatory cytokine, IL-10. This is a cytokine that acts primarily to inhibit the inflammatory response, produced by many cell types (Th2 cells, DCs, monocytes, B cells, and Tregs) [81]. This immunomodulatory effect has also been assessed in human clinical studies (see Effect of probiotics on allergy). Indeed, the consumption of LGG was shown to increase IL-10 production in the serum of children with AD [82].

Finally, it has been shown that two probiotic species belonging to the same genus may have antagonist effects on each other. For example, *L. casei* subsp. *alactus* alone has been characterized during in vitro culture cell experiments as an inducer of proinflammatory cytokines in DCs. However, this effect was not observed with a mixture of *L. casei* subsp. *alactus* and *Lactobacillus reuteri* [83]. This antagonistic effect of a probiotic mix has also been observed in animal models. The administration of *L. casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* in mice increased the systemic production of IgG1, typical of a Th2 response, but *L. acidophilus* alone enhanced the production of IgG2a, typical of a Th1 response [84].

In conclusion, if different probiotic species have equivalent effects on innate immunity by improving mechanisms of pathogen destruction, their effects appear variable (sometimes antagonist) on the adaptive immune system as a function of strains.



**Figure 2. Prebiotic and probiotic effects on the innate and adaptive immune responses.** A nonspecific antiviral effect through the stimulation of innate immune cells: increase in the cytotoxic activity of NK cells and macrophage phagocytosis. As a function of the probiotic strains, pro (Th1 cells)- or anti (Th2 cells and Tregs)-inflammatory responses of the adaptive immune system can be activated.

### Immunomodulatory properties of prebiotics

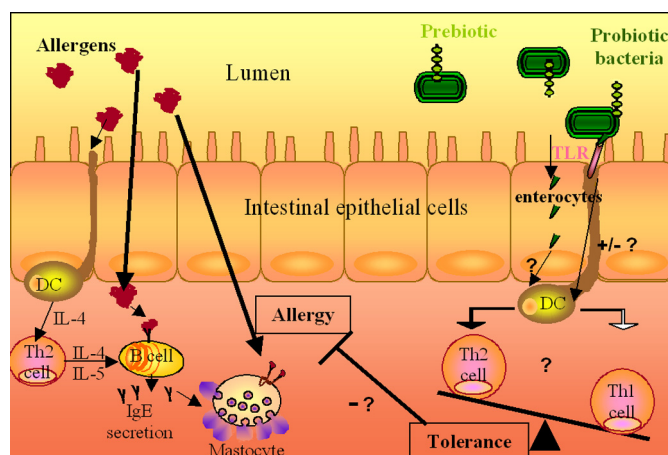
Prebiotics can exert an effect on the intestinal barrier and gut immune system that is mediated by the SCFAs produced by microbiota. One of these SCFAs, butyrate, has been the most studied. Butyrate has been identified as a modulator of histone

tail acetylation, and consequently, it can increase the accessibility of many genes to transcriptional factors (activator or repressor) [85]. The transcriptional effects of butyrate on different subsets of cells involved in the gut immune system have been studied in vitro: neutrophils [86], enterocytes (up-regulation of NOD2 expression in Caco-2 cells) [87], and DCs (impact on DC maturation) [88]. It was thus observed in enterocyte models that butyrate enhanced the intestinal barrier by regulating the assembly of tight junction proteins [89]. All of these studies were performed in vitro on cultured cell lines, but the transcriptional effect of butyrate has also been demonstrated in vivo in humans. Vanhoutvin et al. [90] established that the local administration of butyrate resulted in an increased transcription of the genes mainly associated with energy metabolism and inflammation (TNF- $\alpha$  signaling). Butyrate is produced by the *Clostridium*, *Eubacterium*, and *Ruminococcus* genera, whereas other SCFAs, such as acetate or propionate, are produced by lactic acid bacteria of the *Bifidobacterium* and *Lactobacillus* genera. As mentioned above, prebiotics stimulate the activity of lactic acid bacteria much more than butyrate-producing bacteria. So butyrate alone cannot explain the effects of prebiotics on the gut immune system; propionate and acetate must also play key roles in the regulation of expression of immune system genes. Indeed, Cavaglieri et al. [91] showed that lymphocytes harvested from LNs and cultured with acetate, propionate, and butyrate produced high levels of IL-10, whereas butyrate alone had little effect. Kiefer et al. [92] demonstrated that propionate significantly modulates histone acetylation in HT-29 cells.

### EFFECTS OF PROBIOTICS, PREBIOTICS, AND SYNBIOTICS ON ALLERGY

The intestinal epithelium is constantly exposed to a multitude of foreign materials that can be harmful or beneficial to the organism. Consequently, the intestinal immune system needs to find a balance between the protective immune responses that are induced following an encounter with intestinal pathogens or toxins and the tolerance of commensal bacteria and food antigens. Inadequate protective immune reactions can cause severe immune disease and are actively prevented in healthy individuals. Food antigens and commensal bacteria constitute the majority of the antigen load in the intestine, and the “default” reaction of the immune system confronted with them leads to systemic unresponsiveness. This phenomenon is known as oral tolerance and is a key feature of intestinal immunity [93]. The result of interactions among intestinal contents, unique anatomical features, and immune and non-immune cells is an environment that favors the tolerance by the induction of IgA antibodies (by TGF- $\beta$ -secreting Th3 cells) and CD4<sup>+</sup> Tregs (producing IL-10 and IFN- $\gamma$ ). This ensures that a homeostatic balance is maintained between the intestinal immune system and its antigen load, so that it retains the ability to recognize dangerous and harmless antigens as foreign and preserves the integrity of the intestinal mucosa. The inappropriate immune responses to food and commensal bacteria that are responsible for celiac disease, Crohn’s disease, and food allergy are a result of a deregulation of these crucial

processes [94, 95]. In ~6% of children and 4% of adults in the United States, tolerance of a given dietary antigen is not established or breaks down, resulting in food hypersensitivity. Although food allergies can result in sudden and life-threatening symptoms, their prevalence is remarkably low considering the complexities of the gut-associated mucosal system [96]. An allergic reaction mainly corresponds to the activation of Th2 cells against food allergens and occurs in two phases (Fig. 3): the first phase corresponds to transport of the allergen through the intestinal barrier, its capture by APCs (DC or enterocyte), and its presentation to naive Th0 cells, which differentiate in the presence of IL-4 into Th2 cells. Activated Th2 cells then produce an IL-4 cytokine that enables the production of allergen-specific IgE by B cells [97]. These secreted IgEs then bind to mast cells via the IgE receptor Fc $\epsilon$ RI. The activation phase corresponds to the degranulation of mast cells after further exposure to the same allergen that links directly with specific IgE on the surface of these cells. This phenomenon triggers release of the allergic mediators involved in clinical manifestations of allergy. The gut microbiota plays a crucial role in the establishment of tolerance to food antigens. Indeed, studies about axenic mice revealed a failure in the acquisition of tolerance to some food proteins [98]. Differences in terms of microbiota genus and species have been observed between allergic versus healthy individuals in some studies. Indeed, He et al. [99] have found major differences in the fecal flora of these two populations in terms of the profiles of bifidobacteria species and their ability to adhere to human intestinal mucus (more adherent in healthy children). By monitoring the gut flora of allergic and healthy children from two different countries (Estonia and Sweden), Björkstén et al. [100] evidenced a lower colonization by anaerobic bacteria (bifidobacteria) and elevated counts of some aerobic species (coliforms) in children developing an allergic tendency at the age of 2 years. Thus, modifications to gut microbiota in allergic individuals through the use of food supplements such as probiotics or prebiotics could in the future constitute an inter-



**Figure 3.** Description of the sensitization and activation phases of the allergic reaction. Prebiotic and probiotic effects on the Th1/Th2 balance; an impact on allergy?

esting strategy to prevent or treat allergic diseases. Indeed, at present, the standard of care for food allergy includes a strict avoidance of food allergens and ready access to self-injectable epinephrine. The problems involved in avoiding food allergens and the potential for sudden and life-threatening reactions can diminish the health-related quality of life of patients and their families.

### Effect of probiotics on allergy

Numerous animal and human studies have been performed to test the potential effects of different probiotic strains, mainly in the context of preventing and treating allergies.

#### Probiotics as tools for allergy prevention

**Animal models** Most studies have been performed in animals with distinct genetic backgrounds: different mouse strains [68, 101–105], rats [106], and dogs [107]. In most cases, the probiotic effects were assessed in adults (mice aged 6–8 weeks). Fewer studies have focused on the influence of perinatal administration (given to the mother or pups). The effectiveness of some probiotic strains in preventing allergy has been shown in many studies by an increase in the Th1 response (*Escherichia coli* Nissle [101], *Lactococcus* [102], several bifidobacteria and lactobacilli [103]); an alleviation of clinical symptoms (*Lactobacillus* [104]); and an increase in TLR2 expression (*Lactobacillus* [68]). However, negative effects of probiotics on allergy have also been evidenced [105, 106]. Indeed, de Jonge et al. [106] showed that *L. casei* Shirota administered to 3- to 4-week-old Brown Norway rats before allergen sensitization increased the Th2 response and the number of basophilic granulocytes in the blood. Yang et al. [105] observed that live *Bifidobacterium adolescentis* and *Bifidobacterium longum* administered to Balb/c mice increased DTH and spleen lymphocyte proliferation. It therefore appears that not all probiotic strains have the same effect on preventing allergy: only some of them are effective and can achieve prevention, and others stimulate the allergic response.

We consider that these heterogeneous effects of probiotics can also be explained by the diversity of study methods, in terms of the differences in parameters, such as the genetic background of animals, their age, the probiotic strains used, the state of bacteria (live or heat-killed), or the sensitization mode.

**Human studies** Human studies can be considered separately as a function of the perinatal conditions of probiotic administration. The probiotics were given only to the children (five studies) [108–112] or to mothers and children (five studies) [113–117]. Under the first condition of administration (children only), it is impossible to reach a clear conclusion as to the effect of the probiotic, as all of the potential impacts on allergy were observed: an increase in allergy [112], no effect [109–111], or a clear decrease [108]. Of the five studies using a combined approach (mothers and children), the majority appeared to suggest a protective effect on allergy [113, 115, 116] with a reduction in the incidence of atopic eczema. However, the analysis of the cohort developed by Kalliomäcki et al. [113] in the longer term (2–4 years [118] and 7 years [119]) showed that the probiotic effect was not sustained over the long-term. Moreover, the study of Kopp et al. [117], which

had been developed with the same experimental protocol as Kalliomäcki et al. [113], did not demonstrate any effect of the strain at short-term. The same percentage of children developed an allergy, and there was no difference in IgE concentrations. They also observed that children with recurrent episodes of wheezing bronchitis were more frequently in the probiotic group.

Various strains of probiotics have been used during studies in humans and animals, but that most studied has been LGG [110, 113, 117]. Surprisingly, although administered under the same conditions, this strain seems to have contradictory effects. Thus, of the two studies about LGG administration in mothers and children [117, 118], only one [118] established a slightly positive effect of LGG on allergy prevention at 6 month of age (reduced risk of atopy). Moreover, when LGG was administered in children only, Scalabrin et al. [110] saw no preventive effect.

Consequently, even if the combined approach (mother+ children) has enabled more positive results in the short-term than other modes of administration, insufficient data are available to affirm that probiotics can prevent allergy in humans.

In conclusion, the heterogeneity in the design of animal and human studies (different strains, doses, periods of exposure, and environmental conditions) could explain the contradictory effects of probiotics on preventing allergy. However, individual parameters are also likely to be responsible for these disparities, such as the environment, the composition of microbiota, or genetic background, etc. Thus, probiotics cannot yet be considered to constitute effective tools to prevent allergy.

#### Probiotics as tools for the treatment of allergy

**Animal models** Only three studies [120–122] have been carried out using animal models. They involved the use of mice of various ages (3–8 weeks) and different probiotic species alone or combined by intranasal or oral ways after allergen sensitization [*Lactococcus lactis*, *L. casei*, *Bifidobacterium breve*, *B. infantis*, *Bifidobacterium animalis*, *L. plantarum*, *L. rhamnosus*]. These studies evidenced a reduction in the allergic response, characterized by a decreased Th2 response (reduced production of specific IgG1, IgE, and IL-4) and an increased Th1 response (augmentation of TNF- $\alpha$  and IFN- $\gamma$  production).

Despite their disparities in terms of protocol design, the results of these studies were consistent with a beneficial therapeutic effect of probiotics. Nevertheless, further studies are necessary to confirm the interesting effects of this food supplement and to explain the mechanisms involved.

**Human studies** Human studies about probiotics as a treatment for allergy have revealed varying impacts: a beneficial effect of probiotics causing a decrease in Severity Scoring of Atopic Dermatitis Index levels in patients who received probiotic-supplemented formulas [123–126], a moderate effect [127–129], or no effect [130]. *L. rhamnosus* sp and particularly, LGG are still the most widely studied probiotics [124, 125, 127–130]. The effectiveness of this latter probiotic strain on the treatment of AD has not yet been assessed. Indeed, only two studies have described a clear improvement in allergic symptoms [124, 125]. Finally, in a setting of AD, Brouwer et al.



[130] did not demonstrate any significant effect of LGG on its symptoms, inflammatory parameters, or cytokine production.

To conclude, although animal studies have described beneficial effects of probiotics in the treatment of allergy, the results of human studies have not, so far, permitted any clear conclusion as to the effectiveness of probiotics, especially on longer term.

### Effect of prebiotics on allergy

The effects of prebiotics on allergy prevention and treatment have been less studied than those of probiotics. Most studies have been performed in a preventive setting, and only one using an animal model focused on testing the efficacy of prebiotics in a therapeutic context.

**Animal models** All animal studies have been performed in mice, particularly using those with a Balb/c genetic background [47, 131, 132]. Various prebiotics were used: GOS [47], FOS [132, 133], or the GOS/inulin combination, with or without pectin-derived acidic oligosaccharides [131]. All studies showed a preventive effect of prebiotics on allergy. Fujitani et al. [133] observed an antiallergic effect of FOS, which was characterized by a reduction in mast cell levels and in the rate of edema formation in the duodenum when NC/Jic mice were sensitized with OVA. Watanabe et al. [132] demonstrated a reduction in hypersensitivity reactions in Balb/c mice sensitized epicutaneously to 2,4-dinitrofluorobenzene and then receiving feed enriched in FOS. Vos et al. [131] observed that combined GOS/FOS decreased the Th2 response and induced activation of the Th1 pathway when Balb/c mice were sensitized i.p. with OVA. Pirapatdit et al. [47] showed that Balb/c mice, sensitized with OVA and receiving GOS-enriched feed, have fewer chemoattractant cytokines for cells involved in the allergic reaction. All of these studies were performed on adult mice (aged 4–6 weeks when receiving the prebiotic), but it would be interesting to test the prebiotic effect during the perinatal period in the same way as probiotic strategies.

Metugriachuk et al. [134] were the only team to study the effects of prebiotics as a treatment for allergy. They demonstrated a decrease in OVA-specific IgE levels and total IgG levels in mice sensitized to OVA and treated with a prebiotic phyto compound added to milk.

**Human studies** An Italian cohort of infants with a parental history of atopic eczema, allergic rhinitis, or asthma was used to evaluate the possible effectiveness of prebiotics in preventing allergy [135–137]. The prebiotic tested was a mixture of GOS/inulin included in a hypoallergenic formula containing extensively hydrolyzed cow's milk whey protein. The infants were fed the GOS/inulin formula for 6 months, and any signs or symptoms related to allergy (AD, wheezing episodes, and allergic urticaria) were evaluated during the first 6 month of life [136] and then 18 months after the termination of prebiotic supplementation (aged 2 years [135]). At the ages of 6 months and 2 years, the incidence of allergic disease was reduced significantly in this Italian cohort receiving the GOS/inulin formula [135, 136]. In the 6-month infants treated, an increase in bifidobacteria in the feces and a fall in the levels of total IgG1, IgG2, IgG3, and cow's milk protein-specific IgE were observed [136, 137]. Thus early nutritional intervention

with prebiotic oligosaccharides appears to be effective in priming the infant's immune system in a balanced way and ensuring substantial protection against allergy. Moreover, this preventive effect seems to be preserved over the long-term, even after prebiotic supplementation has been stopped.

In conclusion, a prebiotic strategy appears to be more promising than a probiotic strategy in the prevention of allergy, as all animal and human studies have produced encouraging results. This protective effect could be mediated by an increased abundance of autochthonous lactobacilli or bifidobacteria that can interact with the intestinal barrier and gut immune system. Other bacterial genera may also be responsible for the production of immunomodulatory metabolites such as SCFAs. Nevertheless, no definitive conclusion, as yet, can be drawn, as the number of studies performed remains insufficient. It would be interesting to test the effect of different perinatal administration regimens for prebiotics during human or animal studies (mothers, children, or both) to characterize more specifically the effects of prebiotics on autochthonous gut microbiota and on the immune system to then understand their mechanisms of action in allergy prevention.

### Effect of synbiotics on allergy

Two clinical human studies have analyzed the effects of synbiotics in a food allergy context [6, 138]. Their preventive effect was studied by Kukkonen et al. [6], who showed that a mixture of four probiotics associated with GOS significantly reduced childhood eczema but had no effect on other allergic symptoms. However, this effect was not sustained over the long-term [139].

Passeron et al. [138] assessed the effects of synbiotics in the treatment of allergy. They observed a reduction in the clinical manifestations of AD when allergic children (aged 2 years and over) were treated for 3 months with a mixture of *L. rhamnosus* and the metabolites of this bacterium considered to be prebiotics. Nevertheless, in this study, there was no real control group, as only prebiotics alone and synbiotics groups were studied, and both of these groups showed the same effect on AD. Therefore, the effect of preparations remains uncertain.

Only one animal study has tested the preventive effect of the synbiotic *B. breve* associated with GOS/inulin in mice orally sensitized with whey [140]. Schouten et al. [140] demonstrated that this synbiotic markedly reduced the allergic skin response and anaphylactic reactions. It does not affect whey-specific IgE and IgG1 responses, but IgG2a levels were increased.

There are insufficient bibliographical data at present to reach any conclusion as to the effects of synbiotics on allergy. There is also a lack of data concerning the effect of prebiotics on the survival of specific allochthonous probiotic strains in humans or animals.

## CONCLUSIONS AND PERSPECTIVES

The general effects of probiotics on the innate immune system are now well documented (causing an increase in antipathogen activity). By contrast, the amount of data about the spe-

cific effects of different strains on adaptive immunity needs to be improved. Studies have shown that a same probiotic strain can exert different effects on T cell subset activation (Th1, Th2, Th17, Treg-1, etc.). In other words, these immunomodulatory properties seems to be bacteria-specific and environment-dependent. For each probiotic strain, it would be necessary to establish in vitro the profiles of the cytokines secreted by lymphocytes, enterocytes, and/or DCs that come in contact with a strain. This will make it possible to obtain clear data about the pro- and anti-inflammatory properties of the strain in question and to better define preventive or curative clinical protocols for gut diseases. Probiotic strains use dietary fibers (such as prebiotics) as a substrate, so that prebiotics are also interesting tools that could act on intestinal allochthonous (probiotics) or autochthonous (commensal) microbiota. Numerous sources of prebiotics exist, but most studies have been performed using the same mixture, i.e., GOS and inulin. These compounds may increase fecal *Bifidobacterium* and *Lactobacillus* genera in humans and animals, but their specific effects on each strain and on autochthonous commensal microbiota are not documented. There is also a lack of data about other prebiotics. It will therefore be important to characterize more specifically the effects of prebiotics on the growth of autochthonous gut microbiota and the implantation of probiotics.

Because of their immunomodulating properties, probiotics, prebiotics, and synbiotics may constitute valuable tools to treat and prevent immune disorders such as allergy, but as yet, their mechanisms of action are not fully understood. The bibliographical data do not enable any clear conclusion regarding probiotic beneficial effects on the prevention or treatment of allergy. Nonetheless, prebiotics appear to be interesting tools to prevent allergy when they are administered in infants during the first month of life. Further clinical studies will clearly be necessary to determine the better perinatal mode of prebiotic administration. These future studies may enable us to understand different mechanisms, such as the establishment of intestinal flora and its effect on the immune system. The experimental findings available at present on synbiotics are insufficient to reach any conclusion. Moreover, all of the studies performed so far regarding the influence of probiotics and/or prebiotics on allergic diseases were carried out under heterogeneous experimental conditions: different cohorts of children (with an allergic risk or not) and varying clinical protocols (different strains, doses, periods of exposure, and environmental conditions). It is therefore difficult to compare the results of the numerous studies on the impact of these food supplements on allergy. Future data, first on the effects of each probiotic strain on the adaptive immune system and second, on the impact of prebiotics on each autochthonous and allochthonous strain will then be crucial to determine the appropriate strategies for the treatment and prevention of allergy. Homogeneous experimental protocols are essential to be able to compare their results and to conclude as to any positive or negative effects. The major challenge will consist of defining the perfect combination of prebiotic(s) and probiotic strain(s) that can induce tolerance. The ideal doses and administration regimens need to be determined. Much remains to be achieved before gut immune disorders can be prevented or

treated using well-characterized probiotic bacteria strains, specific oligosaccharide prebiotics, or a combination of these.

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## KEY WORDS:

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