

Role of Gut Microbiota in Food Tolerance and Allergies

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Key Words

Gut microbiota • Food intolerance • Food allergy

Abstract

Alterations of commensal flora may cause various gastrointestinal and extraintestinal diseases, including food intolerances and food allergies. According to the 'microflora hypothesis', alterations in the composition of gut microbiota in industrialized countries have disturbed the mechanisms of mucosal immune tolerance. Over the past few years several studies have looked for a role for probiotics in the treatment of food allergies with promising results.

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Gut Mucosal Barrier

The intestine, like the skin, is the largest body surface area that comes into direct contact with the external environment. Because of this, the intestinal mucosa structure requires a complex organization that can provide effective protection from potential pathogens coming from the surrounding environment [1].

The intestinal mucosal barrier has chemical and physical functions that contribute to the proper maintenance of the body, including absorption of nutrients, protection from potentially pathogenic enteric microorganisms and toxins, and maintenance of immunometabolic balance of the host.

The elements that constitute the intestinal mucosal barrier are multiple and structured at different levels. The first is represented by the extensive surface of epithelial cells, the enterocytes, which are closely interconnected by the presence of intercellular junctions that favor the formation of a physical barrier to help block the passage of potentially harmful into the body. These joints include the intercellular tight junctions such as claudin, zonula occludens, occludin, and F-actin; the adherence junctions formed by the interaction of E-cadherin, α_1 -catenin, β -catenin, and F-actin; and desmosomes constituted by the interaction of desmogleins, desmocollin, desmoplakin, and keratin filaments [2, 3]. The epithelial cells are also essential in the immune recognition and act as antigen-presenting cells (fig. 1).

Another class of cells which are located in the epithelial layer is made up of the goblet cells. These cells are located among the enterocytes and are closely linked to the production of mucus, located above the epithelial surface,

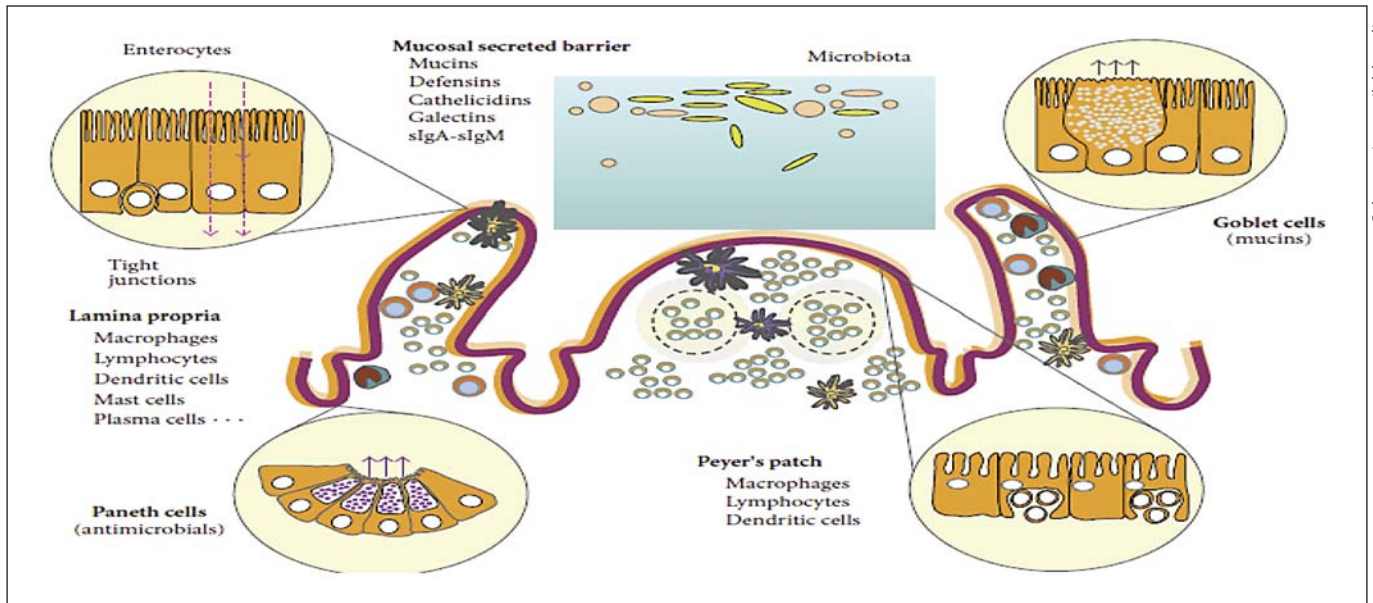


Fig. 1. The elements of the intestinal mucosal barrier. The enterocytes are closely interconnected by the presence of intercellular junctions, which include the intercellular tight junctions such as claudin, zonula occludens, occludin, and F-actin; then by the adherence junctions formed by the interaction of E-cadherin, α_1 -catenin, β -catenin, and F-actin; and desmosomes constituted by

the interaction of desmogleins, desmocollin, desmoplakin, and keratin filaments. The epithelial cells, in addition to the barrier function, are essential in the immune recognition and act as antigen-presenting cells. Reproduced with permission from Bibiloni and Schiffrin [53].

responsible for protection of the host. Thus, goblet cells constitute a physical barrier together with enterocytes and the mucus layer. The intestinal mucus is synthesized in the goblet cells, and then secreted in the intestinal lumen. The mucus is located directly above the mucosal cells and accounts for two layers. The innermost layer, which is in direct contact with epithelial cells, is the true mucosal barrier. At this level there are also secretory IgA and antimicrobial substances produced by goblet cells and Paneth cells. This is a very compact layer structure in the presence of MUC3 [4]. The outer layer is formed with mainly commensal microorganisms that have direct contact with the intestinal lumen (fig. 2).

Another important component of the intestinal mucosal barrier is the complex system of intercellular interactions regulating the mucosal immune system. The main actors of this system are secretory molecules, produced mainly by Paneth cells including secretory IgA, defensins, mucin, cathelicidin, and galectin, as well as many others in the intestinal lamina propria such as macrophages, lymphocytes, dendritic cells, and Peyer's patches. Moreover, M cells located at the surface of epithelial cells are specialized in antigen recognition and serve as anti-

gen-presenting cells, exogenous antigens leading to dendritic cells for subsequent immune activation.

Luminal factors contributing to the establishment of the intestinal mucosal immune barrier are mainly represented by the commensal microbial flora.

Gut Microbiota

At birth the fetus is sterile, not having had contact with any type of bacterial flora during pregnancy. At the time of delivery, it comes in contact with the vaginal bacterial flora. In addition, dietary and environmental factors contribute to form the 'core native microbiota', whose composition is more or less stabilized within the first year of life. The practice of cesarean delivery has a key role in influencing the composition of gut microbiota of the newborn. These changes are associated with an increased risk of developing a food allergy in infants delivered by caesarean section [5–7]. The microbial flora is different across individuals and may change in the same individual depending on the stages of life. Among the factors that most influence its characteristics that we have found are

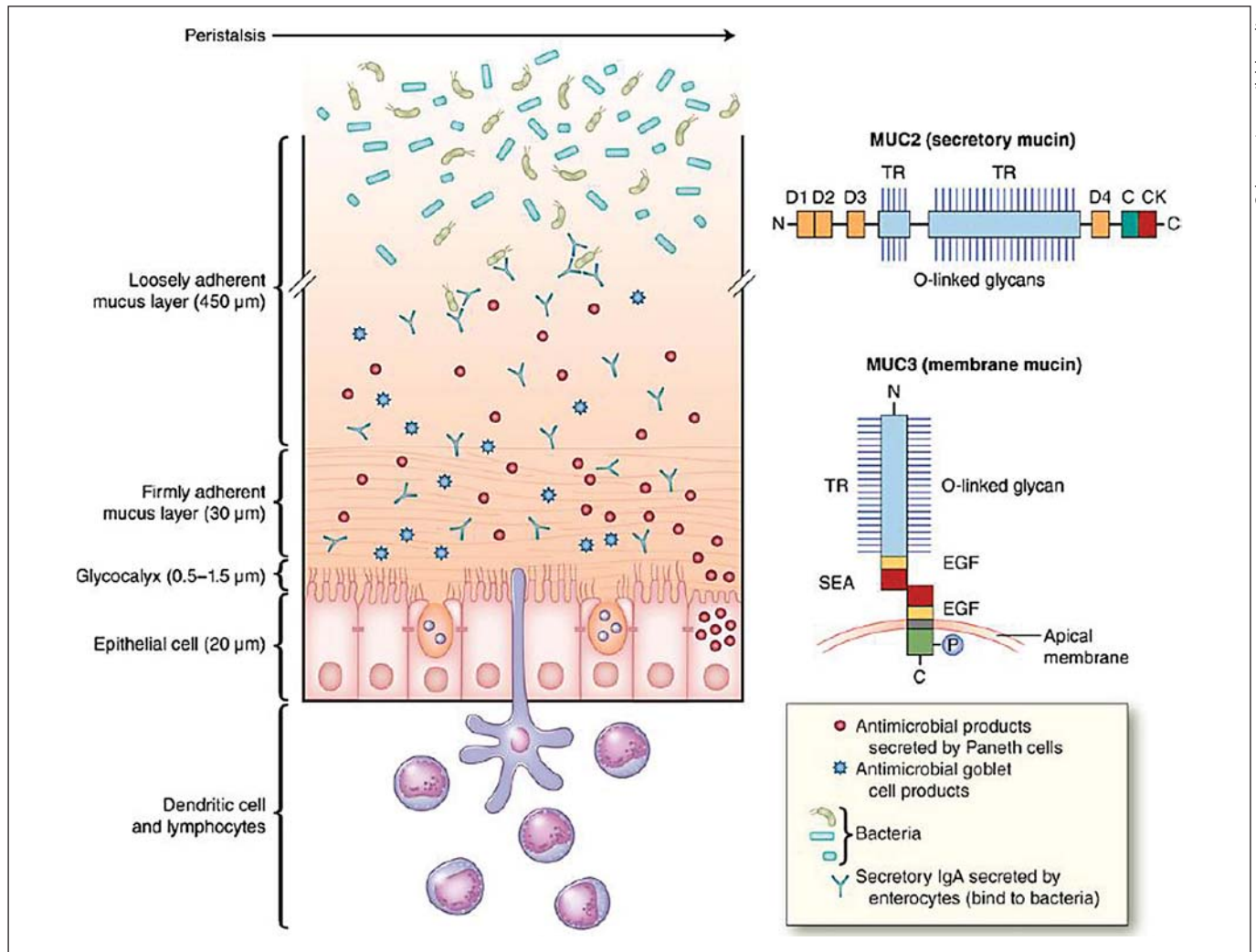


Fig. 2. The goblet cells are located among the enterocytes and are closely linked to them, producing the mucus located above the epithelial surface. Moreover, they contribute to the formation of a physical barrier for both the interconnection with enterocytes and the layer of mucus; therefore, the mucus is located directly above the mucosal cells and forms two layers. The innermost lay-

er, in direct contact with epithelial cells, is the true mucosal barrier. At this level there are also secretory IgA and antimicrobial substances produced by goblet cells and Paneth cells. The outer layer is formed with mainly commensal microorganisms that at this level have direct contact with the intestinal lumen. Reproduced with permission from Kim and Ho [4].

lifestyle, physiology and pathology of the host, genotype, environment, and characteristics of competence of the immune system.

As evidence of the interaction between the microbiota and the mucosal immune system, the switch and maturation of the immune system from neonatal to adult life may be taken as an example. The fetus during intrauterine life has an immune system that favors a Th2 response. This characteristic is essential to ensure the growth of the fetus in the womb without triggering an immune re-

sponse that would lead to abortion. The gut microbiota plays a fundamental role in the development and programming of the mucosal immune system in the newborn [8]. The Th2 phenotype leads to the predominant synthesis of IgE by B cells with increased risk of developing allergic diseases through the activation of mast cells. Microbial stimulation during early life promotes the development of the Th1 phenotype, thus creating a new budget. Th1–Th2 and Th3 cells stimulating the activity of regulatory functions is mainly through the synthesis

of TGF- β which favors the switch for IgA secretion and inhibits the synthesis of IgE [9]. Their combined action leads to the exclusion of food allergens and reduce the exposure of antigens to the mucosal immune system (fig. 3) [10].

Experiments on mice have shown the crucial role of commensal bacteria in establishing and regulating the intestinal barrier. The beneficial effects of these bacteria appear to be mediated by their recognition through pattern recognition receptors, including Toll-like receptors and Nods, located on the epithelial surface. Their ligands are the microbial associated molecular patterns. After recognition, polarized epithelial cells are able to activate intracellular mechanisms for activating NF- κ B, which causes the release of defensins against potential pathogens. This protective mechanism appears to be finely regulated by cytokines, produced in the lamina propria by dendritic cells and macrophages, resulting in the control of inflammatory reactions of autoimmune nature. If the antigenic load increases excessively or if immune regulation fails, hypersensitive reactions and autoimmunity can result (fig. 4).

Gut microbiota also has a role in immunocompetence and tolerance. The major component of the mucosal immune system is represented by the gut-associated lymphoid tissue, representing approximately 70% of lymphoid tissue. Twenty-five percent of the intestinal mucosa is made up of lymphoid tissue and consists of three main compartments which have several morphological and anatomical differences. The major constituents of lymphoid tissue are lymphocytes, plasma-cells, macrophages infiltrating Peyer's patches, and lymphoid follicles. Gut microbiota stimulate the mucosal immune system through the maturation of the gut-associated lymphoid tissue, which occurs predominantly in the small intestine. The commensal microflora is able to promote the development of innate immunity due to mucosal up-regulation of transcription of genes for the synthesis of defensins, lysozymes, chemokines, secretory IgA, and bactericidal proteins. All pathogenic microbes are composed of molecular structures recognized at the mucosal immune barrier, called pathogen-associated molecular patterns (PAMPs). PAMPs are essential polysaccharides, polynucleotides that differ among pathogens but not found in the host. These include flagellin for flagellated bacteria, peptidoglycan for Gram-positive bacteria, lipopolysaccharide for Gram-negative bacteria, and β -glycan for yeast [11].

Intestinal microbiota are directly involved in the development of innate and acquired mucosal immune re-

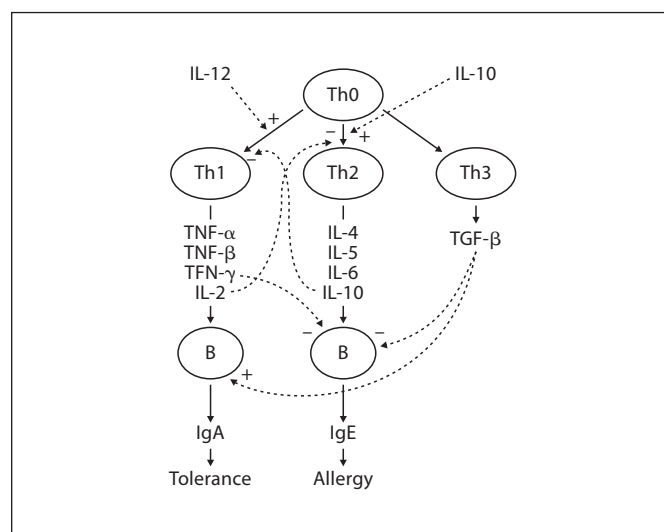


Fig. 3. The gut microbiota plays a fundamental role in the development and programming of the mucosal immune system in the newborn. At birth, in fact, the infant's immune system is not fully developed and tends to have a phenotype predominantly of the Th2 type to prevent the phenomenon of rejection in utero. The Th2 phenotype, however, leads to the predominant synthesis of IgE, increasing the risk for allergies. Microbial stimulation during early life promotes the development of the Th1 phenotype, creating a new budget. Th1–Th2 and Th3 cells stimulate the activity of regulatory functions mainly through the synthesis of TGF- β , which favors the switch for secretion of IgA B cells and inhibits synthesis of IgE. Their combined action promotes the synthesis of IgA, contributing to the exclusion of food allergens, and reduces the exposure of antigens to the mucosal immune system. Reproduced with permission from Ouwehand [8].

sponse. This stimulation occurs through the receptors at the enterocyte surface, which are called pattern recognition receptors and divided into receptors for the recognition of extracellular antigens (Toll-like receptors) and receptors for the recognition of intracellular antigens (Nods).

The gut microbiota also have metabolic, synthetic, and processing functions in close liaison with the human body's metabolic processes. They are excellent energy anaerobic bioreactors, and they can consume, store, and redistribute the energy produced. The gut microbiota also allow us to extract energy from substances not otherwise useful in terms of energy, such as indigestible carbohydrates. Intestinal microflora are able to use the substances consumed in the diet: bacteria can transform complex polysaccharides and monosaccharides in short-chain fatty acids. Short-chain fatty acids are a source of energy for colonocytes and directly affect the storage of

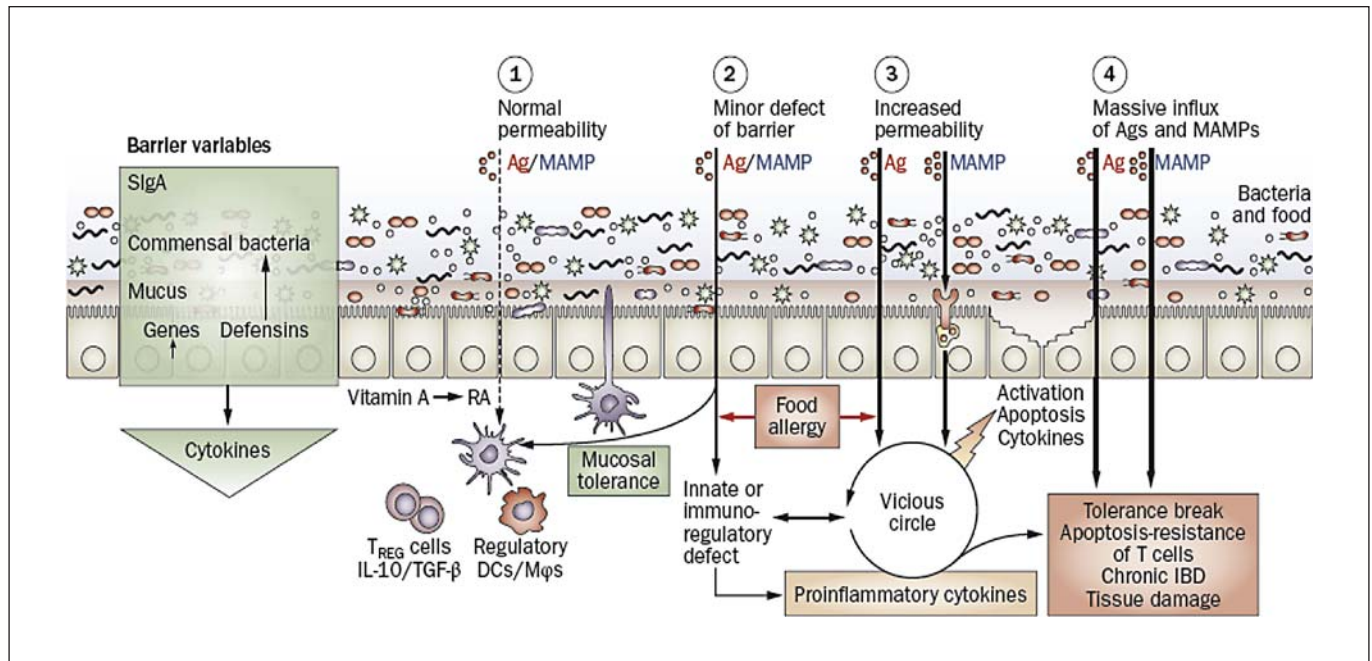


Fig. 4. The intestinal homeostatic balance is maintained between all the elements at various levels, and these are involved in the processes of tolerance and immune response. The beneficial effects of commensal bacteria appear to be mediated by self-recognition through receptors called pattern recognition receptors, including Toll-like receptors and Nods, located on the epithelial surface. The ligands that are recognized are the microbial associated molecular patterns. Polarized epithelial

cells are able to activate intracellular mechanisms to activate NF- κ B, with release of defensins. This protective mechanism appears to be finely regulated by cytokines. Therefore, when the responses of control are fairly low, antigenic loads that consistently reach the lamina propria are controlled by the response. However, if the antigenic load is too high, hypersensitivity reactions and autoimmunity could result. Reproduced with permission from Brandtzaeg [3].

lipids and the absorption and metabolism of food, creating the so-called 'second meal effect'.

Qualitative and quantitative alterations of commensal flora may result in various gastrointestinal and extraintestinal diseases. Food intolerances and allergies are an emerging entity in the microbial related diseases universe.

Gut Microbiota and Adverse Food Reactions

Adverse health effects arise from an immune or non-immune response that occurs reproducibly upon exposure to a given food. Within this group, it is possible to distinguish the disease from toxins and food hypersensitivity reaction. These can in turn be distinguished from immune-mediated diseases, i.e. food allergies (which can be divided into IgE-mediated, i.e. occurring within 2 h of exposure to the allergen; and non-IgE-mediated), and nonimmune-mediated, i.e. food intolerance (fig. 5) [12].

Food allergies account for only a small percentage of all adverse reactions to foods and their prevalence has increased over the past 10–15 years, particularly in industrialized countries: 3–6% of children under 3 years of age and 1–3% of adults. Food allergens in children are represented by milk, egg, wheat, soy, peanuts, tree nuts, fish, and shellfish. The majority of allergic processes that develop during the childhood tend to abate with age, whereas those that occur during adulthood tend to persist. Hypersensitivity refers to an excessive immunological reaction to food antigens with undesirable consequences [3].

In IgE-mediated food allergies, the trigger for the immune response requires binding of the specific allergen to specific IgE located on the cell surface of macrophages and mast cells. This binding activates intracellular mechanisms with release of preformed mediators, including histamine, tryptase, prostaglandin D₂, and leukotrienes. Food allergies present with respiratory, gastrointestinal, and cardiovascular symptoms. The IgE level and the skin prick test are important for diagnosis. Cell-mediated re-

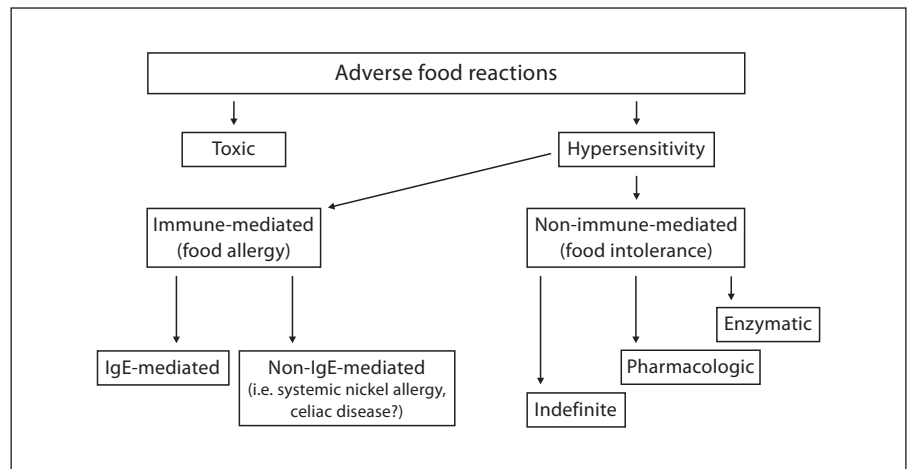


Fig. 5. Classification of adverse food reactions. Reproduced with permission from Patriarca et al. [12].

actions include a broader class of diseases represented by the dietary protein-induced enterocolitis syndrome and proctocolitis. They are characterized by the onset of vomiting and diarrhea 2 h after ingestion the offending food, such as milk, soy, or wheat. There are also mixed syndromes represented by atopic dermatitis and eosinophilic gastroenteropathy; 35% of children with moderate-to-severe atopic dermatitis have food allergies [13].

Factors directly involved in developing food allergy are usually divided into host factors and those related to the antigen characteristics. The host factors include genetic predisposition, age of exposure to the allergen, food disruption of the normal intestinal barrier (leaky gut), quantitative alterations of the gut microbiota, and the use of antacids. The factors related to food allergens include binding to conformational or sequential epitopes, nature of the antigen, route of exposure, and type of antigen-presenting cell involved [13].

Food allergies can be commonly defined as adverse immune reactions to food proteins that can cause an immune reaction ranging from immediate and potentially life-threatening to chronic disorders such as atopic dermatitis and allergic gastrointestinal disorders [14]. Food allergies are caused by an aberrant Th2 immune response against common innocuous food antigens (allergens) in susceptible individuals [3].

Allergic reactions appear to be predominantly Th2. A sequence that can mimic the activation of an allergic process could be as follows: stress, stimulation of bacteria, and cytokines can destroy the intestinal mucosal barrier allowing excessive passage of antigens through the mucosa in the lamina propria. The immunological environment is characterized by a predominant activation of

dendritic cells and basophils. The preferential activation of Th2 cells leads to IgE production and infiltration of mast cells. Mast cells produce inflammatory mediators such as histamine, prostaglandins, and leukotrienes in response to surface binding of IgE with the allergen, and also promote the recruitment of eosinophils and the constriction of smooth muscle [15].

Role of Gut Microbiota in Food Allergies: The ‘Microbiota Hypothesis’

The fundamental importance of the gut microbiota in engaging in this type of pathology can be summarized by the ‘hygiene hypothesis’ showing that the cleaning measures adopted in modern society reduce the exposure of children to adequate stimulation by environmental bacteria. The changes in hygiene, diet, and medical practices have altered the bacterial exposure and the composition of the gut microbiota. In addition, other factors may play a role in the hygiene hypothesis: smaller families, improved hygiene, vaccination, and the consumption of more and more sterile food, thereby reducing exposure to bacteria [16, 17]. The hygiene hypothesis is based on the concept that insufficient exposure to environmental microorganisms is a cause of development of allergies [18]. This hypothesis is supported by two major areas of evidence: the increased incidence of allergies in the last 30 years and the difference in the prevalence of allergies in Western countries and developing countries. This change in habits has deprived infants of adequate immunological stimuli and has changed the composition of gut microbiota [3].

For the reasons above, some authors derived the concept of the 'microbiota hypothesis'. The concept is that alterations in the composition of gut microbiota in industrialized countries (due to the use of antibiotics, changes in lifestyle, and food in particular) have disturbed the mechanisms of mucosal immune tolerance. This concept is supported by an ample body of evidence. First, there is a positive correlation between increased risk of allergies and increased use of antibiotics [19]. Secondly, there is a correlation between atopy and changes in gut microbiota: (1) it is difficult to induce immunological tolerance in germ-free animal models, (2) PAMP administration together with food antigens increases the effect of tolerogenic food, and (3) some bacterial toxins can break immune tolerance [20]. Moreover, changes in diet or the use of probiotics can provide effective prevention or a reduction in allergies [21].

A recent study by Rodriguez et al. [22] studied the correlations between signs and symptoms of allergies and alterations of gut microbiota in a mouse model of cow's milk protein allergy. In brief, conventional and germ-free mice were sensitized orally with wheat protein and cholera toxin or with cholera toxin alone (control group). Subsequently, both groups underwent oral challenge with β -lactoglobulin and the allergic response was evaluated with clinical symptoms, markers of plasma awareness, and values for T helper Th1/Th2/T_{reg}. Composition of microbiota was assessed by denaturing gradient gel electrophoresis and culture. The germ-free mice were more responsive to the sensitization than conventional mice, and awareness did not alter the composition of gut microbiota.

The relationship between alterations in gut microbiota and allergies was also assessed in humans. First, it is known that the gut microbiota of atopic subjects is different from that of healthy controls [19]: the condition is associated with atopic increased levels of aerobic bacteria and low levels of lactobacilli [23]. Additionally, in the fecal microbiota of allergic infants, higher levels of Clostridia and reduced levels of Bifidobacteria, and Enterococcus and Bacteroides were found [24]. In addition to these quantitative differences, qualitative differences were also observed: infants with atopic dermatitis had a more 'adult' microbiota, with a high prevalence of *Bifidobacterium*; in contrast, microbiota of healthy infants is more frequently populated by *Bifidobacterium bifidum*, typical of breast-feeding [8, 25]. Finally, it was demonstrated that bifidobacteria in infants suffering from atopic dermatitis induces a higher secretion of proinflammatory cytokines, while those of healthy infants induce a higher secretion of inflammatory cytokines [26].

The endogenous flora is essential in the development of oral tolerance. It is estimated that specific PAMPs can be recognized by antigen-presenting cells and can stimulate the immune system towards development of a controlled immune response.

The change in the type of endogenous flora, and the ability to induce the production of a specific cytokine pattern favors the maintenance of immune homeostasis in autoimmune and autoinflammatory diseases. An example is the mouse model of inflammatory bowel disease in which mice with colitis are colonized by *Helicobacter hepaticus*. When colonized with *Bacteroides fragilis* they do not show the inflammatory disease pattern. The mechanism of protection against inflammatory bowel disease, mediated by the presence of the *B. fragilis*, is believed to be mediated by the release by the same protein surface antigen [27]. The production and secretion is recognized and taken up by intestinal dendritic cells, which present it to naive CD4⁺ T cells. This leads to the activation of IL-10 secretion by T cells of a potent anti-inflammatory cytokine able to block the proinflammatory processes mediated by other factors such as the secretion of IL-17, or triggered by bacteria such as *H. hepaticus* [28].

In the context of adverse reactions to foods another great chapter is represented by not immune hypersensitivity to food: the food intolerances. Intestinal absorption of carbohydrates requires activation of luminal and mucosal intestinal enzymes. At the level of the brush border, there are more than 22 enzymes, including peptidases and disaccharidases, and more than 19 receptors and transport systems for the final absorption of simple carbohydrates [29]. The cause of possible food intolerance is represented by a deficiency of these enzymes: deficiency of lactase results in lactose intolerance, and the lack of a membrane carrier determines fructose, sorbitol, and trealose malabsorption. The main symptoms of sugar intolerance are abdominal pain, bloating, flatulence, and diarrhea.

Food intolerance can be primary, but in several patients is derived from an underlying cause. In most cases this is represented by the intestinal bacterial overgrowth, a clinical syndrome characterized by bacterial overgrowth or bacterial translocation in charge of the small intestine with consequent impairment of the mechanisms of digestion and absorption of carbohydrates. In fact, once they arrive in the intestinal lumen, carbohydrates are not digested by the brush border disaccharidases and are improperly fermented by bacteria in small intestine lumen resulting in increased gas production [30].

Role of Probiotics in Food Allergies

Over the past few years, several studies have searched for a possible role of prebiotics and probiotics in the treatment of food allergies with positive, but preliminary, results. Gut microbiota is essential in the development of food allergy in early life, in that the immune system such as the intestinal mucosal barrier has not completed its development. Gut microbiota, in fact, play a crucial role in the induction of oral tolerance as shown in mice raised in a germ-free environment not developing tolerance [31]. In addition, oligosaccharides in breast milk lead to an increase of flora dominated by bifidobacteria and lactobacilli in infants [32]. Conversely, allergic mothers have a lower content of bifidobacteria in their milk, and this condition has a direct impact on the composition of the microbiota of children [33].

There is evidence that probiotics interact with the mucosal immune system via the same pathways as the commensal flora [34]. Marschan et al. [35] have shown that in children with an allergic tract, the use of probiotics induce an increase in plasma levels of IL-10 and total IgA. In addition, allergic infants treated with probiotics have low levels of fecal IgA (considered a marker of maturation of the immune system), which is associated with a lower risk of atopy in the first 2 years of life [36]. The role of mucosal IgA is to neutralize the luminal antigenic load, also participating in the antigen transport from the gut lumen, thus decreasing the transition into the other tissues. So far, probiotics seem to reduce the antigenic load from the intestinal lumen [37].

Another possible route of action of probiotics is the ability to maintain a low degree of mucosal inflammation that supports tolerance to antigens [37]. In fact, probiotics stimulate innate immunity and induce low-grade inflammation that can cause downregulation of allergic immune responses: the use of *Lactobacillus* GG in children with cow's milk protein allergy has led to a significant increase in CRP and IL-6 [38], and treatment with probiotics in children with food allergies have shown an increase in the IFN- γ response of monocytes after serum stimulation in vitro [39]. Probiotics may 'normalize' the functions of the intestinal barrier (mucosal dysfunction can lead to aberrant antigen absorption), thus preventing the colonization by a pathogenic microflora (fig. 6) [40].

Several probiotics play a role in immune responses through immune modulation, prompting the development of T_{reg} rather than favoring a proinflammatory immune response. For example *Lactobacillus paracasei* inhibits the secretion of Th1 and Th2 cytokines, leading to

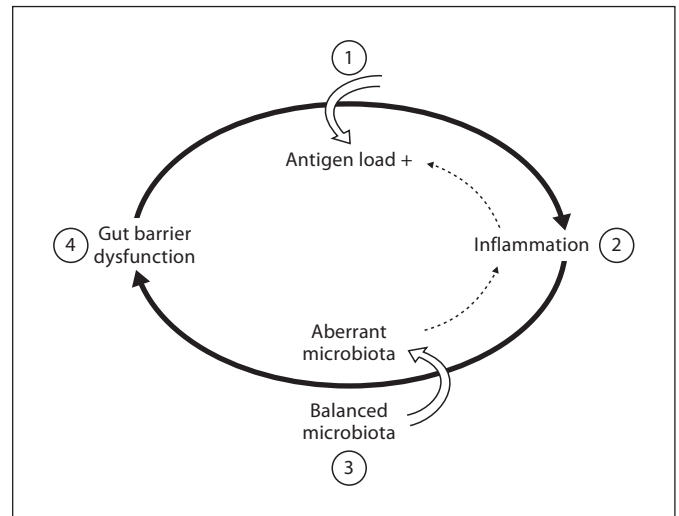


Fig. 6. Intraluminal antigens drive a logistic reaction that damages the intestinal barrier and aberrant antigen absorption in sensitized subjects. Probiotics could act degrading antigen load, stopping the inflammatory response, avoiding deviant microbiota development, and repairing the gut barrier dysfunction. Reproduced with permission from Isolauri et al. [40].

the development of a population of CD4⁺ producing TGF- β and IL-10, regulatory cells involved in immunological tolerance and 'homeostasis' of the digestive system [9]. Moreover, *Lactobacillus reuteri* and *Lactobacillus casei* stimulate dendritic cells derived from monocytes with development of T_{reg} that produce IL-10 [41]. The DNA of Bifidobacteria induces the secretion of IL-10 by mononuclear cells in the plasma of healthy subjects [42].

The clinical data regarding the use of probiotics in food allergies are promising. Adding *Bifidobacterium lactis* Bb-12 and *Lactobacillus rhamnosus* GG led to faster recovery from atopic dermatitis (2 vs. 6 months) compared to standard treatment with *B. lactis* Bb-12 or *L. rhamnosus* GG alone [43]. The combination of *L. rhamnosus* 19070-2 and *L. reuteri* DSM 122460 significantly reduced the clinical score of atopic dermatitis in children 1–13 years of age with a positive skin prick test. In more than half of the subjects, there was an improvement of eczema, compared with the control group [44]. Additionally, *B. BGN4 bifidum* inhibited the production of serum IgG1 and IgE in a mouse model of food allergy, and *L. casei* Shirota, which stimulates the secretion of IL-12, suppresses IgE-specific antigens. Finally, Torii et al. [45] have shown that oral administration of *L. acidophilus* L-92 inhibits the production of IgE specific for ovalbumin.

On the other hand there is a set of concepts and body of evidence regarding the use of probiotics in food allergies that raise several issues. Abrahamsson et al. [46] have recently shown that *L. reuteri* does not alter the incidence of atopic eczema in infants compared with the control group when given to the mothers before birth or in the first 12 months of age. Kuitunen et al. [47] used a group of probiotics (*Lactobacillus* GG, *L. rhamnosus*, *Bifidobacterium* short, and *Propionibacteria*) administered to mothers with a high risk of having allergic children and to infants for 6 months, resulting in no significant difference in the frequency of allergies with the control group after 5 years of follow-up. A review of the Cochrane library in 2007 assessing the outcomes of the use of probiotics in allergies and/or hypersensitivity to foods showed

insufficient evidence to recommend supplementation with probiotics in the diet of infants for the prevention of allergies and/or hypersensitivity to foods.

There are several considerations that may help to better elucidate this discrepancy among the available body of evidence. First, different results may result from different study populations [48] and strains of probiotics [49]. Indeed, a key factor in the body's response to the stimulation provided by microbial probiotics is the age of the subjects studied as demonstrated both in animal and human models [50]. Moreover, strain-specific effects have been shown since the earliest studies [51]. Finally, the transport of antigens by probiotics is affected by the food substrate (e.g. the protein quality of food) [52].

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