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# Intestinal barrier dysfunction and food allergy

**Edited by the SIAIP Food Allergy Commission**

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## SUMMARY

The dramatic increase in allergic diseases has been explained by different hypotheses over time. Most recently, the epithelial barrier hypothesis argues that environmental exposure to toxic substances (detergents, microplastics and nanoplastics, food additives such as enzymes and emulsifiers), a consequence of the modern lifestyle, damage the epithelial barrier of the skin, airways, and intestinal mucosa leading to the onset of various diseases including food allergy. Numerous studies have shown a link between increased intestinal permeability and food allergy. Increased intestinal permeability, by facilitating exposure to allergens, triggers the activation of an immune response involving epithelial cells and immune cells and causes an abnormal Th2-type response. A key role is also played by the gut microbiota and ultimately by diet in determining the proper functioning of this barrier. Opportunities for intervention to repair the damage of the epithelial barrier at present consist of the use of certain components of the diet such as small peptides contained within hydrolyzed formulas, prebiotics, probiotics, vitamins, and fiber that can positively influence barrier function by acting directly on the integrity of the barrier itself, or indirectly through modulation of the gut microbiota. Other strategies such as microRNAs, small molecules, and transplantation of the gut microbiota are under investigation.

**KEYWORDS:** epithelial barrier, food allergy, environmental pollutants, microbiota

## INTRODUCTION

Food allergy (FA) is an immune-mediated adverse reaction to food proteins caused by an inability of the immune system to develop tolerance after exposure to them or a loss of an already established oral tolerance. It is a condition that affects about 5-8% of children and 2-4% of adults and has been increasing in recent decades [1]. Numerous factors have been pointed to as the cause of the increase in FA found especially in Western countries in recent decades, such as increased pollution, hygiene, drug use, psychological stress, decreased physical activity, and changes in diet [2]. However, it remains to be clarified whether these have a direct effect on the host or an indirect one, triggered for example by changes in the composition of the microbiota. Pothoven and Schleimer first proposed the "epithelial barrier hypothesis" in 2017 to explain the increase in many chronic non-communicable diseases observed in

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recent decades [3]. It attributes the origin of these diseases to a defect in the epithelial barrier resulting from environmental exposure to certain substances, including detergents, emulsifiers, food additives, microplastics, and increased ozone levels. Alterations to this barrier have been described in conditions such as bronchial asthma, chronic rhinosinusitis, allergic rhinitis, atopic dermatitis, eosinophilic esophagitis, celiac disease, and inflammatory bowel disease and are central to the pathogenesis of these diseases [4]. A damaged epithelial barrier is associated with colonization of opportunistic pathogens and loss of commensals in organs affected by microbial dysbiosis. An immune response against the dysbiotic microbiome then develops, leading to chronic periepithelial inflammation to expel the microbiota that has moved between and under the epithelial barriers. Overall, a vicious cycle develops with the continuation of mucosal and/or skin inflammation playing a role in the chronicity and exacerbation of the above diseases [4]. Recently, Akdis has proposed to extend this hypothesis to allergic diseases, arguing that the increase in the latter, as well as in autoimmune diseases, is a consequence of the presence of agents resulting from industrialization and urbanization that damage the epithelial barrier [5]. The disruption of the epithelial barrier, brought about mainly by the destruction of tight junctions (TJs) with the consequent increase in epithelial permeability that allows the access of microbes and pollutants, leads to an alteration of the Th1/Th2 balance and a prevalence of a Th1 or Th2 response depending on the cytokines that are produced. In the presence of the interleukins (ILs) IL-4. IL-5, IL -13, IL-9 and IL -6 there will be a Th2 response resulting in an allergic reaction [6]. Epithelial barrier dysfunction appears to precede the development of allergic sensitization and type 2 inflammation. In this paper, we will examine the physiology of the epithelial barrier of the gastrointestinal tract and the causes of its damage resulting in loss of its function and the subsequent allergic pathology, as well as possible therapeutic interventions.

## INTESTINAL BARRIER PHYSIOLOGY AND FUNCTION

The epithelial barrier is present in numerous parts of the body (gastrointestinal tract, urogenital, respiratory, skin, eye) and, in addition to the specific task performed by the epithelium of each apparatus, has a physical and immunological protective function against environmental factors (pathogens, irritants and allergens). In particular, the intestinal barrier is not only responsible for the digestion and absorption of nutrients, electrolytes, and water, but is also a key component interacting with the external environment. Impairment of the gut barrier resulting from exposure to environmental pollutants, foods, chemicals, and changes in the composition and function of the gut microbiota plays an important role on the development of numerous gastrointestinal (chronic inflammatory bowel disease, irritable bowel syndrome, colorectal cancer) and non-gastrointestinal (obesity, diabetes, neurological disorders, and food allergies) diseases that have been shown to be associated with altered gut barrier permeability [7]. The gut barrier is a fundamental component of the body's defense system against the external environment. Although located inside the body, the intestinal lumen is the largest surface area that interacts the external environment. It consists of multiple layers, as illustrated in Figures 1a, 1b, 2, and represents a dynamic and responsive interface between the external world and the human internal environment by first and foremost providing protection from external factors and playing a crucial role in maintaining intestinal homeostasis and thus host health. Epithelial cells are held together by a complex junctional system that firmly binds their sidewalls and includes occluding junctions, adherent junctions, and communicating junctions [8] (Figs. 3a, 3b). In addition to being the first line of defense against harmful agents, pathogens and allergens, the intestinal epithelium naturally also has the specific function of digestion and absorption of various nutrients (Figs. 4a, 4b). In these absorption and digestion processes, TJ regulatory mechanisms play a key role, which are complex and not entirely clear. In response to the action of a wide range of cytokines, the structure of TJs is maintained, but the composition of junctional proteins is changed. An important role in this regard is played by tumor necrosis factor $\alpha$ (TNF-$\alpha$), interferon gamma (IFN-$\gamma$), and some interleukins [9] (Fig. 5).

## INTESTINAL BARRIER AND FOOD ALLERGY

The intestinal barrier constitutes the first line of defense against harmful agents, pathogens, and allergens. The microbiota represents, together with mucus, the dynamic part of the intestinal barrier and

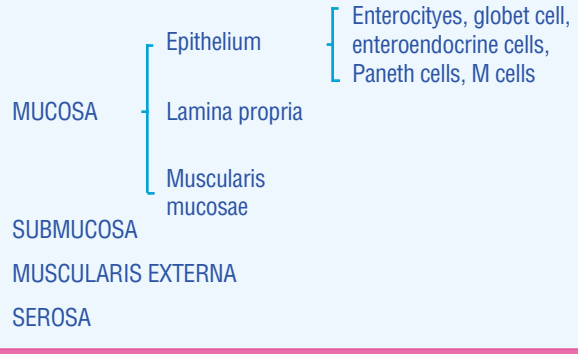


**FIGURE 1a.** Composition of the intestinal wall: the intestinal wall is a complex structure comprising the mucosa, submucosa, external muscular layer, and serosa. Within the epithelial layer of the mucosa, five distinct types of specialized cells are present: enterocytes, goblet cells, enteroendocrine cells, Paneth cells, and microfold (M) cells. These cell populations are continually replenished by a pool of stem cells residing in the intestinal crypts. The mechanical support essential for the integrity of the epithelium is provided by the lamina propria, which is predominantly composed of connective tissue.

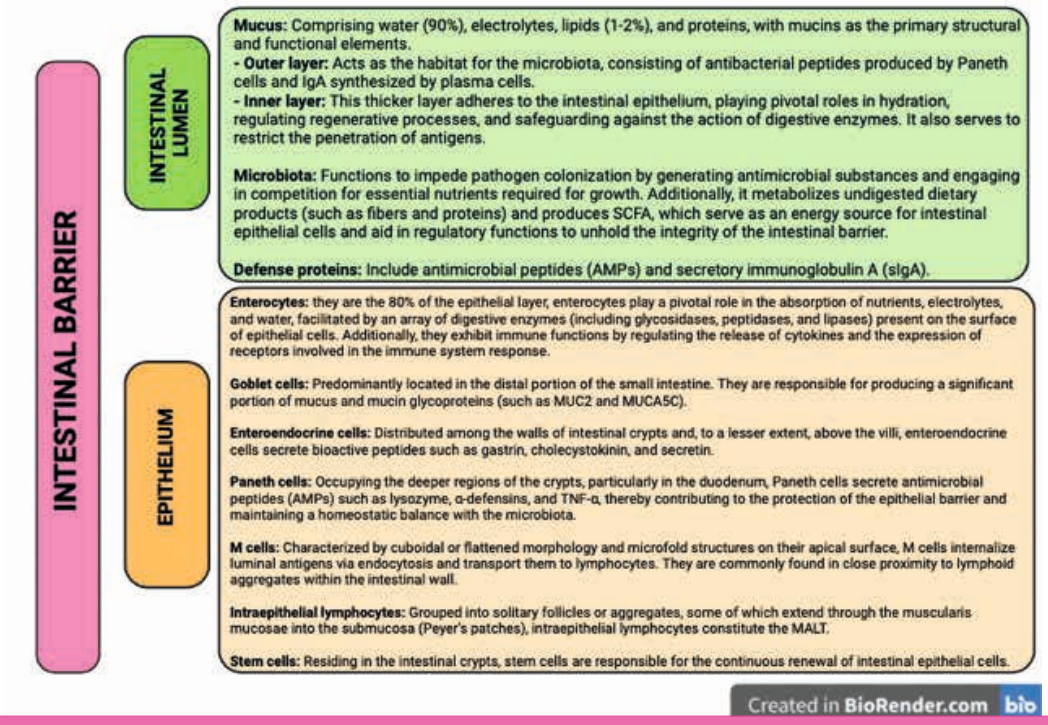


**FIGURE 1b.** Components of intestinal barrier.

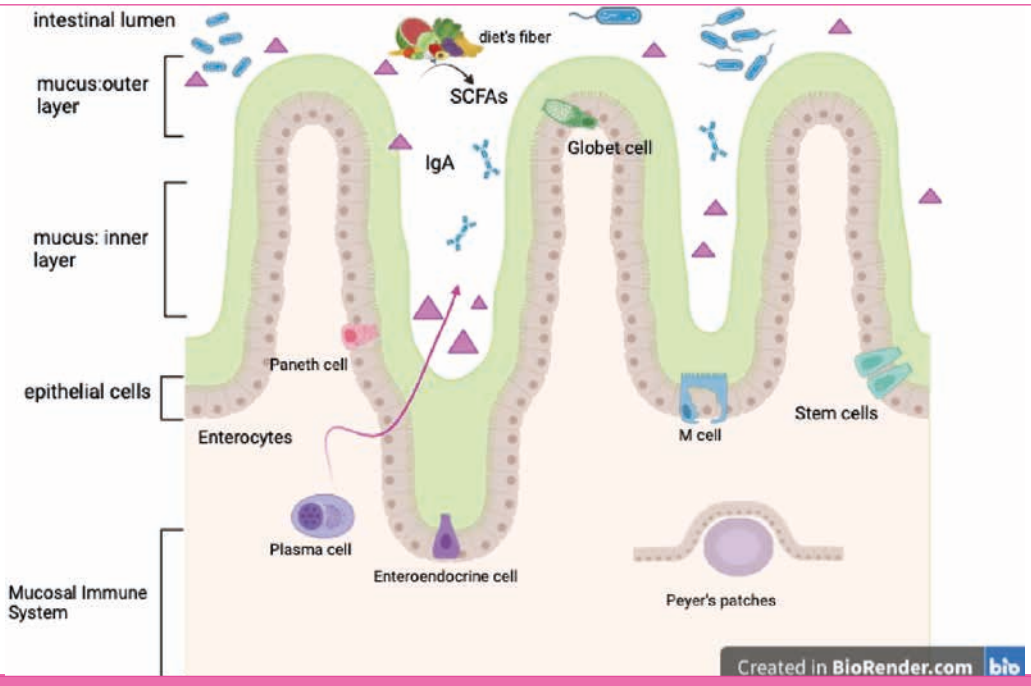


**FIGURE 2.** Composition of the epithelial barrier: The outermost layer encompasses mucus, commensal intestinal microbiota, and defensive proteins such as antimicrobial proteins (AMPs) and secretory IgA (sIgA). The intestinal wall beneath the mucus comprises the mucosa, submucosa, external muscular layer, and serosa (see Fig. 1). The epithelial layer of the mucosa encompasses five distinct types of specialized cells: enterocytes, goblet cells, enteroendocrine cells, Paneth cells, and microfold (M) cells, which are replenished by a pool of stem cells residing in the intestinal crypts. Enterocytes constitute 80% of the epithelial layer and play a pivotal role in the absorption of nutrients, electrolytes, and water, facilitated by an array of digestive enzymes (glycosidases, peptidases, and lipases) concentrated on the surface of epithelial cells.

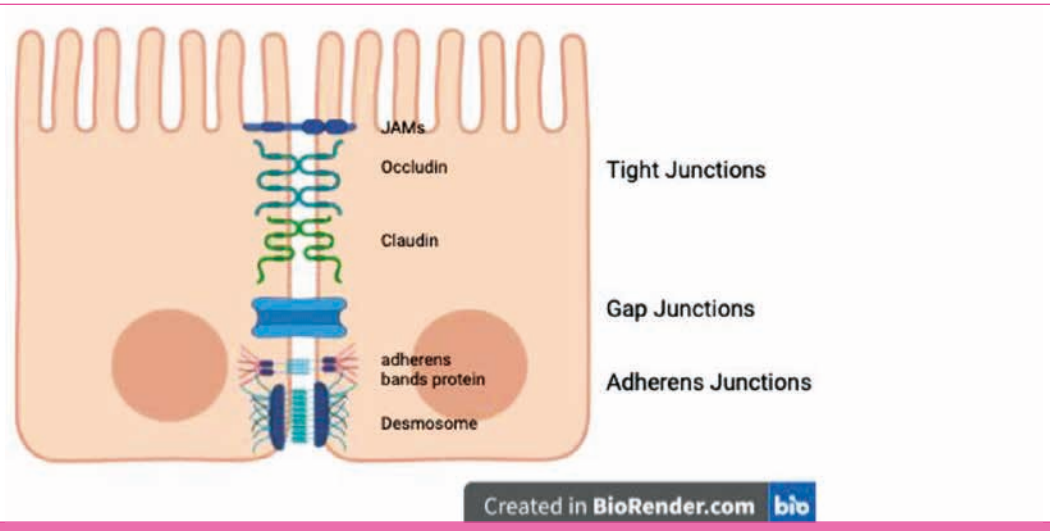


**FIGURE 3a.** Tight junctions (TJs) are crucial occluding junctions located on the apical-lateral surface of epithelial cell membranes play a pivotal role in membrane integrity and selectivity. These intricate structures comprise multiprotein complexes consisting of diverse transmembrane proteins, namely claudins, occludins, junctional adhesion molecules (JAMs), cingulin, and tricellulin. These proteins extend outward from the cell membranes, interconnected by covalent bonds, thus forming a circumferential belt (zonula) around the cell perimeter. Within the cell, their intracellular domains engage in intricate interactions with each other and with proteins belonging to the zonula occludens (ZO) family, notably ZO-1, ZO-2, and ZO-3, which associate with actin filaments, essential components of the enterocyte cytoskeleton. Through these interactions, occludins, claudins, JAMs, and tricellulin collaborate with ZO proteins to preserve the integrity of TJs and regulate the passage of particles across the pericellular space. The primary function of TJs lies in the prevention of substances from the intestinal lumen from infiltrating into the intercellular spaces (i.e., the space between adjacent cells), or conversely, to impede substances residing in the intercellular spaces from translocating into the lumen.

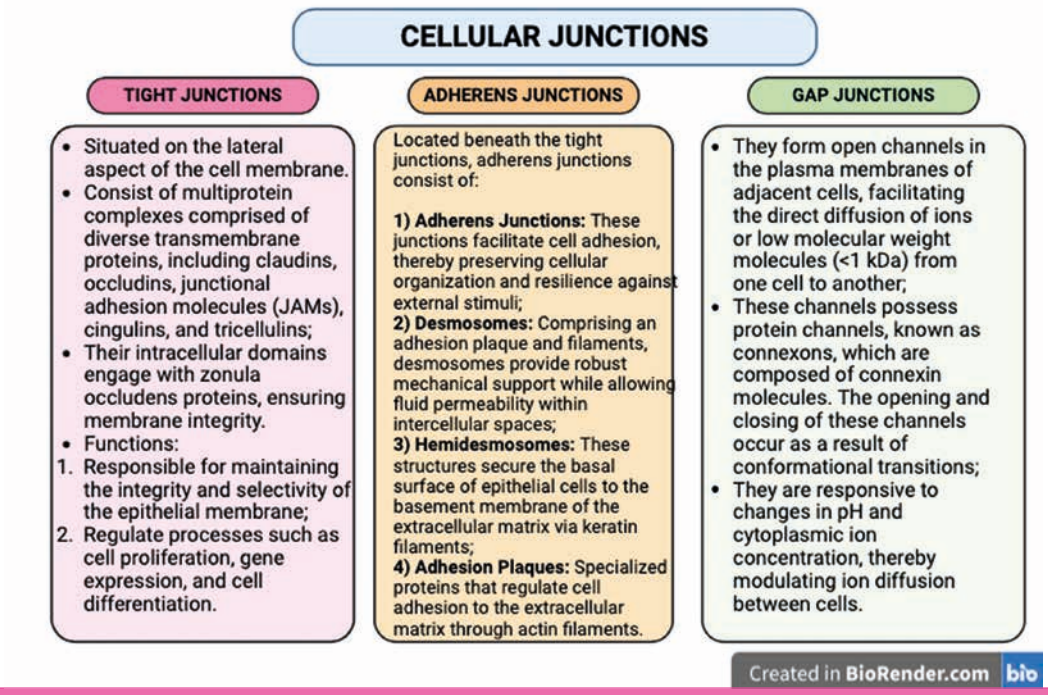


**FIGURE 3b.** Characteristics and functions of cellular junctions.

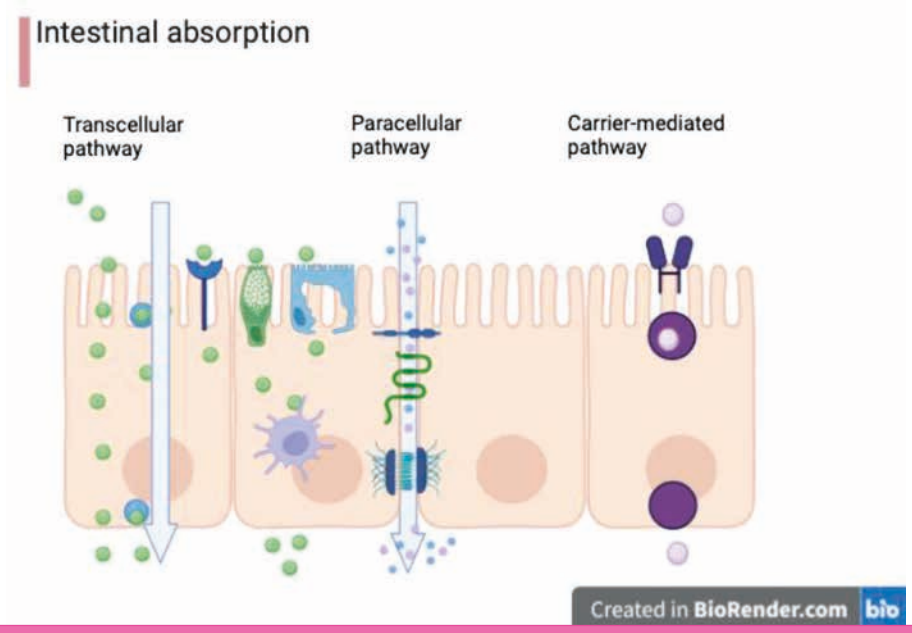


**FIGURE 4a.** The primary function of the intestinal epithelium resides in the selective absorption of water, electrolytes, and nutrients, facilitating their transit from the intestinal lumen into the systemic circulation. The transportation of molecules across the epithelial layer ensues through three principal pathways: a transcellular route, characterized by passive diffusion across cellular membranes; a carrier-mediated pathway, which involves the mediation of carriers/receptors within the transcellular path; and the paracellular pathway, wherein molecules passively diffuse between adjacent cells. Notably, large antigenic molecules, lipophilic compounds, and nutrients traverse through the transcellular pathway, enabling their conveyance across intestinal epithelial cells via mechanisms such as endocytosis, passive diffusion, or binding to specific membrane transporters. Furthermore, transcellular transportation may also take place through M cells, goblet cells, and dendritic cells. Conversely, ions, particularly cations, and small hydrophilic molecules (<600 Daltons) predominantly utilize the paracellular transport route.

is involved in the body's metabolic, nutritional, and immunological processes. The microbiota participates in defense against harmful pathogens through various mechanisms such as resistance to colonization and production of antimicrobial compounds and is also involved in the development, maturation, and maintenance of gastrointestinal sensory and motor function. Commensal bacteria in the intestinal lumen also metabolize indigestible products of the diet such as fiber and protein. Through anaerobic fermentation of complex carbohydrates, they produce short-chain fatty acids (SCFA) such as butyric, propionic, and acetic acids, which provide an energy substrate for intestinal epithelial cells and are involved in numerous regulatory functions by modulating, for example, the expression of occludin and zonulin (ZO), both of which are involved in maintaining the integrity of the intestinal barrier [10]. The intestinal barrier is also present in the intestinal epithelium and subepithelial area. These include both innate and adaptive immune system cells. The cells of the innate immune system constitute the first line of immunological defense in the intestine and are represented by dendritic cells (DCs), macrophages, neutrophils, mast cells, and eosinophils, along with rapidly intervening natural killer (NK) cells [11]. Epithelial cells act as first-line sensors of the epithelium's contact with microbes and can convert bacterial-derived signals into antimicrobial and immune-regulatory responses. Indeed, they express pattern-recognition receptors (PRRs), i.e., receptors that recognize molecular profiles for direct interaction with the microbial environment that enable them to participate in a specific mucosal immune response. PRRs are in fact representative pattern-recognition receptors with a transmembrane protein form that is capable of recognizing pathogen-associated molecular patterns (PAMPs).

The innate immune cell response is triggered by activation of PRRs, such as Toll-like receptors (TLRs), which are alarmed by pathogen-associated molecular patterns (PAMPs), microbe-associated molecular patterns (MAMPs), and damage-associated molecular patterns (DAMPs) present on the surface of pathogenic bacteria. The primary role of TLRs is to recognize bacterial, viral, or fungal pathogens that could be deleterious to the host organism. Activation of TLRs leads to the activation of innate immune cells and the release of antimicrobial compounds, inflammatory mediators, and enzymes. The expression of TLRs and other types of PRRs such as NOD-like receptors (NLRs) and RIG-like receptors (RLRs) on intraepithelial cells

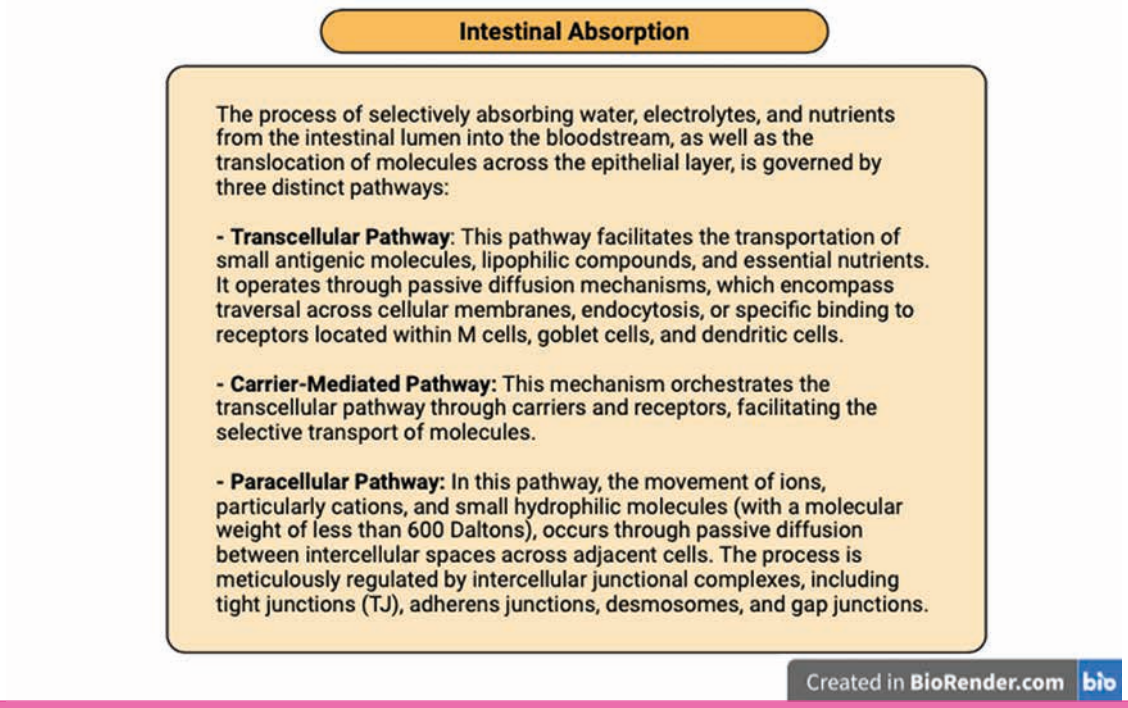


**FIGURE 4b.** Main intestinal absorption pathways.

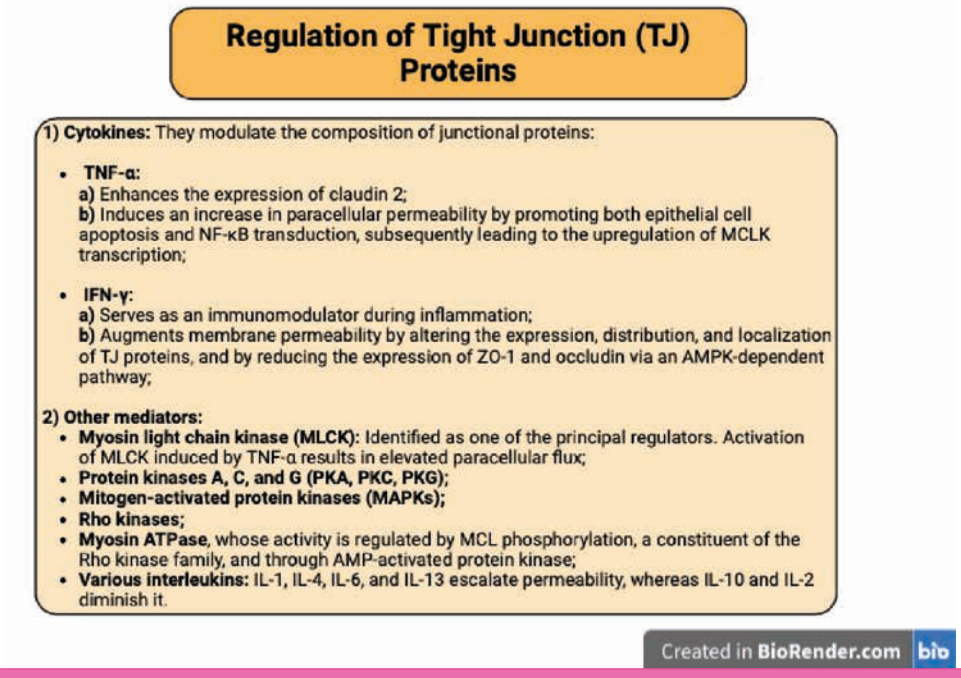


**FIGURE 5.** Regulation of tight junction proteins.

provides a distinct pathway for the recognition of microbial ligands and endogenous signals associated with the disease process [12]. Under physiological conditions, most dietary protein is digested into small peptides and amino acids and taken up by enterocytes. A very small percentage of intact proteins can still pass by endocytosis into the epithelial cells, but are degraded by lysozymes and lose their

antigenic property. Numerous studies have shown a link between increased intestinal permeability and food allergy. Increased intestinal permeability allows penetration of the allergen through the intestinal barrier and consequently stimulates the submucosal immune system. Allergen exposure causes an abnormal Th2-type response, leading to the recruitment of inflammatory cells at the barrier cell level and subsequent tissue inflammation, disruption, and remodeling of the barrier. The antigen penetrates through the damaged barrier and thus triggers the activation of an innate immune response involving epithelial cells and resident immune cells. In this phase, the antigen is captured by dendritic cells and presented to naive T cells, inducing their differentiation into T helper 2 (Th2) cells. Th2 cells promote the production of IgE-producing B-cells whose receptors are expressed on the surface of mast cells and basophils. At this stage, a pool of B-cells and Th2 cells is created [13]. Further exposure to the same antigens causes cross-linking between antigens and mast-cell-bound IgE and leads to immediate activation of an immune cascade, which is rapid, amplified, and effective. Collaterally, this enormous inflammatory response causes tissue damage and a subsequent repair process, which leads to tissue remodeling. There has long been debate as to whether inflammation is the primary cause of barrier dysfunction or whether impairment of the epithelial barrier may favor exposure to allergens and thus trigger an abnormal inflammatory response. It has been shown that in subjects with FA, intestinal permeability increases after challenge and returns to normal after strict elimination diet of the responsible food, indicating that the increase in permeability is a consequence of the allergic reaction [14]. However, other studies have seen that the increase in permeability persists for six months after discontinuation of the elimination diet [15]. It is currently believed that epithelial damage plays a primary role in the pathogenesis of allergic diseases.

## ROLE OF GENETIC FACTORS

Studies have shown that among the genes associated with multiple food allergies is SERPINB10 that is expressed on epithelial cells and involved in the expression of IL-13 in bronchial cells suggesting a role in susceptibility to FA as well [16]. Furthermore, genetic factors may influence the composition of the bacterial flora via TLRs, in particular TLR2, TLR4 and TLR9. In vitro studies have shown that stimulation of TLR9 reduces the production of inflammatory cytokines in polymorphonucleates of allergic subjects [17]. The different localization of TLR9 (on the apical rather than basolateral surface) may be key in driving a more tolerogenic response. Similarly, methylation (or non-methylation) of TLR9 may be an important signal to induce intraepithelial cells to correctly identify healthy microbiota against an invading bacterium and to guide the development of a tolerogenic immune response [18]. Furthermore, TLR2 and TLR9 have been shown to protect against barrier disruption by enhancing ZO-1 expression. In practice, CpG oligonucleotides of bacterial DNA binding to epithelial TLR9 cause an increase in ZO-1 expression, leading to an enhancement of epithelial TJs thus increasing barrier integrity and reducing cytokine production by immune cells. Conversely, activation of TLR4 has an opposing effect.

## ROLE OF ENVIRONMENTAL FACTORS

### POLLUTANTS

In recent decades, the increase in consumption of processed foods, the use of emulsifiers, and the introduction of nanoparticles and microplastics have all significantly increased the burden of environmental exposure on human health. These contaminants can also alter the structure of the gut microbiome and influence the development of allergic diseases by disrupting normal immunoregulation.

#### *Micro- and nanoplastics*

Plastics are widely used every day around the world with global production steadily increasing from 1.5 million tons in 1950 to 348 million tons in 2017. Due to the large production and insufficient recycling, most plastics end up as waste, reaching all ecosystems in the form of microplastics (MP 1-5 µm) and, by further fragmentation, nanoplastics (NP < 100 nm), which pose serious environmental problems, mainly due to their ease of dispersion and persistence [19]. The classification of plastics in the environment is shown in Tables I and II. The oral route, through food and drinking water, is the most frequent route of intake of MPs in humans and other terrestrial living beings, while aquatic organisms can also take them up from the aquatic environment through gill respiration. High concentrations of plastic debris have been found in fish, crustaceans and mollusks and human uptake of MPs has been estimated at 66,000, 28,000 and 36,000 particles per day through the consumption of fish, crustaceans, and molluscks [20] with higher values in countries such as

**TABLE I.** Classification of plastics according to polymer type.

| |
|---|
| High-density polyethylene (HDPE) |
| Low-density polyethylene (LDPE) |
| Low-density polyethylene (LDPE) |
| Polyvinyl chloride (PVC) |
| Polyethylene terephthalate (PET) |

**TABLE II.** Classification of plastics according to origin.

| Primaries | Secondaries |
|---|---|
| Present in:<br>• cosmetics<br>• detergents<br>• paints<br>• hygiene products | Present in:<br>• aquatic environment |

| **TABLE III.** Foods containing microplastics. |
|---|
| Fish products |
| Honey |
| Beer |
| Sugar |
| Water |
| Milk |
| Sea salt |
| Fruits and vegetables |

Belgium, France, and Spain, where crustacean consumption is higher than in other countries such as the UK [21]. In the case of nanoplastics, the risk of uptake may be even higher, due to their smaller size that facilitates passage through the intestinal epithelium and absorption by the body. Studies have detected NPs in human food and drink (Tab. III) and at the 2020 conference of the American Chemical Society, the results of a study were presented in which, for the first time, NPs were detected in human tissue [22]. Once taken in, MPs and NPs pass through the esophagus and stomach, and finally reach the intestinal tract where they can directly damage the intestinal barrier and alter the constitution of the intestinal microbiota. They then pass into the bloodstream and reach other organs with possible toxic effects from accumulation, with the remainder being excreted in feces. Current research has reported that the maximum size limit of microplastics that can pass through the human gut is about 150 µm, and this passage tends to increase as their size decreases. However, particles larger than 1.5 µm rarely penetrate organs and are therefore unlikely to cause organ damage. In addition to size, the type of polymer and the presence of contaminants adsorbed on it can also affect the intestinal toxicity of MPs. In this sense, the major concern relates to the ability of these particles to absorb and interact with a range of organic and inorganic contaminants present in the environment in the form of complex mixtures rather than as individual entities, and even to absorb pathogenic microorganisms that are capable of aggregating and adhering to their surface in complex communities commonly referred to as biofilms. In particular, the pathogens most associated with MPs include potentially pathogenic bacterial species such as *Pseudomonas, Vibrio, Campylobacter* and *Escherichia coli*, which, once ingested, are able to reproduce and exert a toxic action in the body through the production of metabolites and damage the intestinal mucosa, thus compromising the health of the host. It should be emphasized, however, that the available studies on the mixed toxicity of MPs and environmental contaminants have been conducted predominantly on animals, and that the critical evaluation of toxicological findings and potential mechanisms involved in the human gut is not yet sufficiently complete and systematic. Regarding the bidirectional relationship between MP and the gut microbiota, little is known about the microbial degradation capacity of MP in mammals. Indeed, there are currently no studies in humans demonstrating that MP degradation is linked to isolated human microorganisms. However, certain microorganisms in the earthworm gut microbiota are capable of degrading MPs by reducing the size of LDPE (from 150 µm to an average of 53.1-41.3 µm) [23] and it is therefore possible that our gut microbiota may also act on MPs during their intestinal transit, reducing their size and favoring the release of additives or monomers with toxic action.

MPs in the intestine, in addition to causing direct damage, can also cause indirect damage due to the presence of the so-called 'carrier effect', which consists of their ability to absorb and transport other harmful components, such as polycyclic aromatic hydrocarbons, pesticides, chlorinated biphenyls, and heavy metals such as cadmium, zinc, nickel, and lead [24]. Once released, these substances lead to substantial biomolecular, histological and cytological changes in the intestinal mucosa, described in the literature as the 'Trojan horse effect' [25]. In this regard, Fries et al., after selecting MPs from sediment samples collected in Norderney (Germany), were among the first groups to demonstrate the presence of nanomaterials on their surface, in particular titanium dioxide ($TiO_2$) nanoparticles [26]. Nanomaterials are artificially produced microscopic particles with a size of up to 100 nm, which are widely present in water ecosystems and soil in the form of emulsions, polymers, ceramic, metal, and carbon particles. Their accumulation and toxicity have been demonstrated mainly in animals and plants, but not sufficiently in humans. The importance of environmental exposure from $TiO_2$ is because this compound is widely present in confectionery, chewing gum, sweets etc., in some paints and construction cements, and as a coloring agent (E171) in many food products. In the form of nanometric particles, $TiO_2$ may also be present in some cosmetics (body creams and make-up) and in some sunscreen products due to its ability to filter sunlight. Therefore, it is evident that exposure to $TiO_2$ nanoparticles can be manifold, and its absorption can occur preferably through food, but also through skin contact or inhalation. Due to their small size, $TiO_2$ nanoparticles are able to easily cross the gastrointestinal barrier where the alteration of the physical and chemical properties of the food matrix and carrier biopolymers takes place. This can exert an activating action on the innate and adaptive immune response with negative impacts on the intestinal barrier and intestinal microbiome, which are key events characteristic of the pathogenesis of chronic inflammatory bowel diseases. In fact, in vitro studies have shown that, in the intestine, $TiO_2$ nanoparticles are able to destroy TJs between epithelial cells, increase the expression of pro-inflammatory cytokines [27], and significantly reduce mucus secretion. Therefore, due to serious safety concerns, the use of $TiO_2$ as a food additive has been banned from food production in the European Union from January 2022 [28]. In view of the above and the fact that foodstuffs contaminated with MP and NP can disrupt the intestinal barrier and promote an inflammatory environment, the idea that ingestion of these particles can also promote allergic sensitization is quite well-founded and is a widely debated topic. In this sense, although the reported effects have been correlated with exposure to MPs, it is highly likely that NPs may have

the worst consequences since, due to their small size, they can easily cross the intestinal barrier more easily, be internalized and/or alter the biology of intestinal epithelial cells to promote allergic sensitization. Furthermore, by binding compounds with adjuvant activity, they can indirectly promote an inflammatory gut environment and cause intestinal dysbiosis. These conditions may increase the risk of allergic sensitization and contribute to the sharp increase in food allergies observed in recent years. Therefore, in consideration of all these factors, on 16 January 2018 the EU Commission adopted the European Strategy for Plastics in a Circular Economy, which also aims to contribute to the achievement of the Sustainable Development Goals by 2030, particularly those protecting the environment by reducing plastic waste at sea and fossil fuel-related emissions. The aim is not to eliminate plastic altogether, which would be unrealistic, but rather to promote more sustainable plastic production and consumption patterns with the aim of reducing environmental damage and possible consequences on human health.

### *Emulsifying agents*

Today's western lifestyle is leading to profound changes in our diets, which appear to be increasingly characterized not only by a low intake of antioxidants, fiber, and a high fatty acid content, but also by the consumption of foods containing additives such as preservatives, enzymes, and emulsifiers. With reference to emulsifiers, it should be noted that their use has increased significantly in recent years and there is currently an ongoing debate about their safety. Emulsifiers are synthetic substances used by the food industry to make food emulsions more stable over time. The most important are fatty acid mono- or diglycerides, fatty acid esters, polyglycerol esters, calcium/sodium stearoyl-2-lactyl esters, and sorbitan esters, but also many natural derivatives such as lectins, glycolipids, saponins, alcohols, hydrogenated fatty acids, microbial surfactants, etc. These food additives, once ingested, have been shown to alter the composition of the intestinal microbiota, facilitating the presence of bacteria with a potential inflammatory effect and, in addition, to increase bacterial penetration into the body by altering the intestinal barrier [29]. Of particular interest in this respect is the role played by Carrigenin (CGN), a high molecular weight polysaccharide extracted from red algae that is widely used as a food additive for its properties as a thickener, gelling agent, emulsifier, and stabilizer. CGN is normally found in pet food, cosmetics, textile formulations, and products of the pharmaceutical industry as well as in many processed foods, especially those with reduced or absent fat content, such as ice cream, soy milk, yoghurt, salad dressings, beer, cured meats, etc. In addition, CGN is used in the processing of poultry, ham, and red meat food products to increase product yield. In the United States, CGN has been used for many years as a fat stabilizer in infant formula, soy milk, and free amino acid diet formulas or protein hydrolysates used in the feeding of children with cow's milk protein allergies. Several studies, both in vitro and in animal models, have shown that CGN can be dangerous because, during gastrointestinal metabolism, it is degraded into smaller molecular weight components that cause changes in the intestinal epithelium, the intestinal microbiome and, through the activation of NF-kB, the release of pro-inflammatory cytokines, in particular IL-8, all of which can lead to the formation of a new, more toxic and more dangerous protein [30]. A study by Dronen in a peanut-allergic mouse model showed that oral exposure to DON can facilitate the development of anaphylaxis [31]. Despite abundant evidence showing that the consumption of many processed foods is associated with an increased risk of non-communicable diseases, Western countries continue to be the largest consumers of this class of foods. Ultra-processed foods (UPF) are defined as industrial preparations to which five or more ingredients are added, including sugars, oils, fats, antioxidants, stabilizers, preservatives, and additives with the aim of imitating the qualities of unprocessed foods [32]. Emulsifying agents, including carboxymethyl cellulose (CMC) and polysorbate-80, have been shown to have a direct negative effect on the intestinal barrier, leading to metabolic abnormalities of the intestinal barrier and low-grade inflammation in mouse models, abnormalities mediated by modification of the intestinal microbiota [33]. Similar results have been observed for other emulsifying agents. Most of the adverse effects are mediated by the gut microbiota: the consumption of processed food is, in fact, able to increase the expression of virulent factors by gut bacteria, such as lipopolysaccharide, and to reduce the concentration of SCFAs resulting in increased inflammatory status and intestinal permeability and reduction of the mucus layer. In fact, consumption of CMC in humans has shown a significant alteration in the composition of the gut microbiota, a reduction in fecal SCFA levels and may also support invasion by bacteria of the mucus layer.

## Diet

Diet may influence the development of food allergies either by inducing changes in the gut microbiota (leading to the growth or loss of certain bacterial species) or by altering the function of the gut barrier. Regarding the influence on the gut microbiota, a diet rich in fiber appears beneficial in that it facilitates the growth of certain species of fiber-fermenting clostridia that appear to protect against the development of allergies [34]. Animal studies have shown that these clusters of fiber-fermenting clostridia produce SCFA fatty acids, particularly butyrate, propionate, and acetate. The latter stimulate the epithelium and induce hyperplasia of calico cells, increase the production of antimicrobial factors (Reg3b and g), and reduce TLR4 expression. SCFA also inhibit mast cell activation, promote the development of tolerogenic dendritic cells (DC), regulate T-cells, and suppress the DC-Th2-IgE inflammatory pathway [35]. In contrast, a high-fat diet leads to an increase in Bacteroidetes and Delta Proteobacteria and a reduction in bacteria of the genus *Prevotellae*. A cohort study showed that the presence of *Prevotellae* is inversely associated with food allergies [36] and studies in mice showed that a reduction in Firmicutes and an increase in Proteobacteria are associated with an increase and immune response to food antigens [37]. On the other hand, that diet can alter intestinal permeability and the barrier function of the intestinal mucosa is suggested by animal and in vitro studies [38]. Gliadin, the major kiwi allergen (Act D1), and spices and herbs can

alter TJ and epithelial barrier integrity [39]. Studies in mice have shown that a diet rich in lipids and high in sugars can increase transepithelial uptake of antigens [40], reduce mucus layer thickness, and alter the gut microbiota, while polyunsaturated fatty acid supplementation appears to protect and repair inflammatory barrier damage [41]. To date, the allergen exclusion diet represents the cornerstone of FA treatment. Thanks to the effects of food/triggers exclusion, allergic inflammation is then reduced, and this leads to the regeneration of the gastrointestinal mucosa with the consequent improvement of intestinal digestive and absorptive properties. Various dietary components, from small peptides contained within hydrolyzed formulas to actual dietary components such as prebiotics, probiotics, vitamins, and fibers can positively influence barrier function, either by acting directly on the integrity of the barrier itself or indirectly through modulation of the gut microbiota. Other strategies are being studied, such as microRNAs, small molecules and, as the latest frontier, transplantation of the gut microbiota.

### *Hydrolyzed formulas and barrier effects*

Hydrolyzed formulas appear to play an important role in the treatment of cow's milk protein allergy (CMPA). They minimize antigen contact compared to whole-protein formulas, and due to the presence of small peptide fragments, they may strengthen the intestinal barrier through different immunomodulatory mechanisms, such as increasing the expression of anti-inflammatory cytokines (IL-10) or decreasing pro-inflammatory markers, including cyclo-oxygenase 2 (COX-2), NF-kB and IL-8, but also directly, through the expression of genes coding for TJ proteins [42]. The improved barrier function decreases the absorption of the antigen and its contact with intestinal resident immune cells, thus reducing allergic symptoms. Peptides may also exert their immunomodulatory effects through direct stimulation of TLR group receptors present on immune cells that are crucial in achieving oral tolerance, particularly dendritic cells [43].

## GUT MICROBIOTA AND MUCUS: A COMPLEX INTERPLAY

O-glycosylated mucin glycoproteins are the building blocks of the mucus layers and provide lubricating and filtering properties to promote the expulsion of luminal contents and prevent potential pathogens from coming into close contact with the epithelium. It has been shown in ex vivo studies that mice lacking a functional mucus layer (Muc2-/- mice) are more susceptible to colitis and gastrointestinal infections, as well as failure of oral tolerance following sensitization with a food antigen [44]. The gut microbiota is closely related to the composition of the intestinal mucous layer, which is its habitat. Bacteria use glycans to anchor themselves and as a source of nutrition. In this way, the mucus glycosylation profile selects the composition of the microbiota, and, in turn, the microbiota shapes the mucus glycosylation profile, in a complex reciprocal interaction. In in vitro models, it has been shown that mucin itself can play a role as a receptor for dendritic cells in the gut to induce tolerance, especially when MUC2 was administered orally [45].

## PROBIOTICS, PREBIOTICS AND THE ROLE OF SCFA

Probiotics are live microorganisms with proven health benefits. In recent years, a great deal of evidence has accumulated to support their efficacy in improving the tightness and integrity of the intestinal barrier, as they influence the renewal of intestinal epithelial cells through the production of TJs and mucins by tightening cell connections. Probiotics can also modulate intestinal inflammation by promoting the development of the immune system, regulating the release of intestinal antimicrobial peptides, and competing with pathogenic bacteria [46]. This has been the basis for intensive research aimed at identifying the in vivo effects of probiotics. Some studies have demonstrated the effect of specific strains on achieving immunological tolerance [47]. A recent work has confirmed the role of probiotics in promoting tolerance achievement, stressing however that the level of evidence is still low and not sufficient to recommend their routine use [48]. Finally, it must be remembered that the effect is strain-specific and not transferable from one strain to another. It has been hypothesized that the beneficial effect of probiotic supplementation is a consequence of the effects of probiotics on microbiota through the selection of a particular microbial flora, characterized by an increased concentration of *Bifidobacteria* strains and a reduced concentration of *Bacteroides* compared to subjects treated with hydrolyzed formula alone. In addition to quantity, a change in the quality of the *Bacteroides* strains was also observed: the use of probiotics led to an increase in the Bac7, Bac8, and Bac9 oligotypes that are present in the intestines of healthy subjects. These bacterial strains can cause an increase in the concentration of SCFA, in particular butyrate, which in turn has a positive effect on the intestinal barrier. Prebiotics are food components that are not digested or absorbed in the upper gastrointestinal tract and reach the large intestine, where microorganisms selectively utilize them, promoting the growth and activity of selected commensal microbiota in the host. Fiber is a prebiotic that many gut bacteria utilize as nutrition. These dietary compounds mainly include inulin-type fructans (inulin, oligofructose and fructo-oligosaccharides), galactans, galacto-oligosaccharides (GOS), and other heteropolysaccharides such as chitosan, starch, alginate, pectin, and dextran. These components have a positive impact on the function of the intestinal barrier through several mechanisms, including the ability to stimulate selectively the growth and/or activity of the intestinal microbiota, particularly *Bifidobacteria* and *Lactobacilli*. SCFA are the main end products of bacterial fermentation of complex, non-digestible carbohydrates such as dietary fiber. SCFA are carboxylic acids with aliphatic tails of 1-6 carbons, the most abundant of which are acetate, propionate, and butyrate. These exhibit a wide range

of biological functions, including anti-inflammatory responses, modulation of colonic contractility and maintenance of mucosal immune cell activity and intestinal barrier integrity [49]. Butyrate promotes the expansion of mucosal Tregs cells that are crucial in the immunological tolerance network. In this regard, feces from healthy infants and infants with CMPA, before and after treatment with extensively hydrolyzed formula, with or without *Lactobacillus rhamnosus GG,* showed that *Blautia* and *Roseburia* were enriched in the intestinal microbiome of tolerant infants with higher concentrations of SCFA butyrate; this led to the hypothesis that the acquisition of specific strains of these genera is associated with the development of tolerance [50]. Furthermore, under conditions of intestinal homeostasis, the metabolism of colonocytes is deeply dependent on oxidative phosphorylation, which leads to high oxygen consumption. Interestingly, butyrate produced by intestinal bacteria and utilized by the intestinal epithelium affects the $O_2$ levels in these cells, resulting in the activation of the oxygen sensor hypoxia-inducible factor (HIF), a transcription factor crucial for coordinating gut integrity and protection of the intestinal barrier [51]. Finally, butyrate and other SCFAs act at both the TJ level, increasing claudin-2 expression and preventing the partial translocation of occludin from the junctions to the cytoplasm [52] and on mucin production, stimulating MUC2 gene expression in calycephalic cells through acetylation of histones H3 and H4 with a dose-dependent action [53]. Recent studies suggest that butyrate also has a direct effect on mast cells by epigenetically regulating the expression of the FcεRI receptor and inhibiting IgE-mediated degranulation, thus reducing the concentration of histamine, tryptase, TNF-$\alpha$, and IL-6 in the intestinal mucosa [54]. Therefore, butyrate could be used in the future as a possible adjuvant therapy in the treatment of allergic individuals to reduce the risk of reactions. It should be noted that high levels of SCFA butyrate and propionate in feces in children's early years have been associated with protection against food allergies and asthma [55]. While most current investigations of the microbiome in FA have focused on the bacterial microbiota, future research could evaluate the contributions of the viroma and mycobiome. To date, sequencing methods, reference databases and analytical tools for assessing viroma and mycobiome are less developed than those for the bacterial microbiome. However, the refinement of technologies for analyzing data from whole genome sequencing performed on the genomic DNA of a mixed microbial community is enabling research beyond the bacterial microbiome.

## VITAMIN D

Vitamin D is a hormone with pleiotropic effect, whose action at the extra-skeletal level is now widely recognized. It is able to influence the function of several cells involved in the immune response, both innate and adaptive, such as dendritic cells, macrophages, T cells, B cells, and epithelial cells. Vitamin D acts on the expression of various protein components that constitute TJs and adherens junctions, such as E-cadherins and claudin-2, as well as increasing cell migration in vitro, highlighting the central role of this vitamin in epithelium regeneration [56]. Regarding its action at the level of immune cells, this is expressed by increasing the production of IL-10 by macrophages with consequent reduction of TNF-$\alpha$ and by promoting the proliferation of dendritic cells capable of stimulating the proliferation of Treg lymphocytes and with a reduced ability to stimulate against naive T lymphocytes through increasing the CD31 receptor. Vitamin D deficiency can impair the function of the intestinal epithelial barrier and the production of antimicrobial peptides, resulting in an increased risk of intestinal dysbiosis and reduced immune tolerance. Increased intestinal permeability could lead to excessive exposure of trophoallergens to the immune system. In genetically predisposed individuals, such exposure can lead to the development of food allergy. This hypothesis seems to be supported by the results of two prospective studies demonstrating that a state of vitamin D insufficiency at 12 months of age (<50 nmol/L) was associated with an increased likelihood of being affected by food allergy confirmed by oral provocation tests [57].

## FUTURE PERSPECTIVES

### miRNA

MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression by influencing gut homeostasis. In the past decade, the role of miRNAs as modulators of immune response and gut barrier function has been investigated. miRNAs have been implicated in several physiological and pathophysiological mechanisms of the gastrointestinal system and have been extensively studied in immune and inflammatory bowel diseases, including irritable bowel syndrome and IBD, although studies are very heterogeneous. As for the allergology field, all miRNAs that regulate Th1/Th2 cytokine balance and innate immunity response may influence the pathogenesis of allergic diseases. Specifically, inhibition of miR-375 can reduce the development of Th2-mediated inflammation. In addition, suppression of positive regulators of Th2 cell development in CD4+ T lymphocytes, particularly mir-155 and miR-21, can likewise reduce the switch of the immune response toward Th2s. Currently, miRNAs are considered a novel molecular target for the development of new biological drugs. To date, however, there are still a limited number of studies concerning miRNAs mainly due to the complexity and specificity of research.

### GLP-2

A mouse model investigated the role of glucagon-like peptide-2 (GLP-2) in influencing immediate hypersensitivity and allergic inflammation. The authors found that GLP-2 prevents the usual prolonged permeability defect and appears to reduce macromolecular uptake regardless of the antigen uptake pathway and the number of inflammatory cells present in the mucosa [58].

## Mast cell stabilizers

Intestinal mast cells (MCs) play an essential role in the regulation of barrier function and intestinal homeostasis as demonstrated in vitro, in animal models, and in humans. Mast cell activation induces the release of a wide variety of pro-inflammatory and regulatory mediators, many of which influence the intestinal barrier, as well as modulate the immune response and the enteric nervous system. Among mast cell stabilizers, only ketotifen and sodium cromoglycate (DSCG) have been used in clinical practice. There are few studies investigating the effect of mast cell stabilizers in modulating the intestinal epithelial barrier in humans. In one study, ketotifen was shown to be effective in restoring gastroenteric permeability in a small group of patients with FA [59]. Although encouraging, the current clinical evidence is not such that we can conclude for a possible use of these drugs in the routine treatment of FA.

## Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) refers to the transfer of bacterial communities from a donor to a recipient, as has been used for patients with *Clostridium difficile* colitis [60]. An open-label phase I study whose results are not yet available evaluated the safety and efficacy of orally encapsulated FMT for the treatment of peanut allergy (NCT02960074). In any case, the results are entirely preliminary, and important questions remain about FMT for the treatment of FA, including the optimal community to be transferred, the best way to ensure establishment of the donated community, and the safety of the procedure.

# CONCLUSIONS

The rapid increase in the prevalence of allergic as well as other immune-mediated diseases is currently explained as a consequence of epithelial barrier damage. Genetic predisposition, environmental factors, dietary habits, and changes in the composition of the gut microbiota can alter the epithelial barrier and result in local and systemic inflammation underlying diseases such as asthma, allergic rhinitis, atopic dermatitis, eosinophilic esophagitis, and FA. Alteration of the intestinal epithelial barrier has been shown to be strongly correlated with the presence of FA, and it is clear that the functional integrity of the intestinal barrier plays a key role in the prevention of FA. Despite the growing body of evidence supporting the etiological link between intestinal permeability and various diseases, both intestinal and extra-intestinal, the exact mechanisms are still being studied, but they are one of the most promising fields of research for development in the near future. Although pathophysiological, functional, and molecular knowledge has advanced dramatically, and with it the search for therapeutic options to improve intestinal permeability and barrier dysfunction, further translational research is needed that can contribute to the development of new therapeutic strategies and tailored medicine. In addition, it is imperative to raise awareness of the effects of environmental changes on health to motivate those who will influence government policies, for our well-being and that of future generations.

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## Author's contribution

CA, MC: designed the work. All authors gave the same contribution to perform the process of drafting, and the critical revision of the article. All authors gave the final approval of the version to be published.

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