

Review

Intestinal mucus barrier: a missing piece of the puzzle in food allergy

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The prevalence of food allergies has reached epidemic levels but the cause remains largely unknown. We discuss the clinical relevance of the gut mucosal barrier as a site for allergic sensitization to food. In this context, we focus on an important but overlooked part of the mucosal barrier in pathogenesis, the glycoprotein-rich mucus layer, and call attention to both beneficial and detrimental aspects of mucus–gut microbiome interactions. Studying the intricate links between the mucus barrier, the associated bacteria, and the mucosal immune system may advance our understanding of the mechanisms and inform prevention and treatment strategies in food allergy.

In search of mechanisms behind the rise of food allergies

Western countries have been experiencing rising rates of food allergies without a clearly identified cause [1–3], and common food allergies include peanuts, tree nuts, seafood, and cow's milk. Food allergy prevalences in Europe and among US adults were recently estimated at 6% and 10%, respectively [1,2]. Food allergen **sensitization** (see [Glossary](#)) occurs when regulatory immune responses fail to keep up with the induction of allergen-specific proinflammatory immune responses [3]. This drastic increase in food allergy prevalence has led researchers and clinicians to propose that modern lifestyles and environmental changes are a trigger [3]. Identifying the underlying mechanisms that causally connect environmental factors to sensitization is an upcoming area of research that would inform treatment options and prevention strategies.

Food allergies can manifest at all ages, and the large spectrum of symptoms and immune profiles range from immediate IgE-mediated responses to delayed non-IgE-mediated responses [4,5]. Sensitization to allergens and the initiation of allergic disease begin at barrier sites which share key protective features such as a cohesive epithelium that overlies a dynamic immune system ([Figure 1](#)). Atopic march often refers to atopic dermatitis in the newborn, food allergies in childhood, and asthma that can persist as chronic rhinitis into adulthood [6]. Barrier dysfunction leading to the onset of allergy has been documented in the context of atopic dermatitis and asthma where epithelial disruption can be triggered by both genetics and niche-dependent environmental stressors such as pollutants and detergents that compromise barrier integrity [7,8]. At the gut barrier surfaces, multiple interactions directly mirror the causes of sensitization at other organs and mucosal tissues that lead to atopic diseases ([Figure 1](#)) [9].

We focus here on a specific and understudied component of the mucosal barrier in the gut: the gastrointestinal (GI) mucus layer. Mucus is a crucial feature of mucosal surfaces, including the respiratory and digestive tracts ([Figure 1](#)) [10–12]. The mucus layer in the airways plays a protective role similar to that of the digestive tract, and mucus regulation is impaired during disease state such as asthma, causing pathophysiology [13]. O-glycosylated mucin glycoproteins are the building blocks of mucus layers, and provide lubricant and filtering properties to help the expulsion of luminal contents and prevent potential pathogens from entering close contact with the epithelium,

Highlights

The sudden rise in food allergy over recent decades has led to various theories including the role of environmental factors in sensitization.

Gastrointestinal (GI) mucins have recently been implicated in maintaining gut homeostasis: mice lacking a functional mucus layer (*Muc2*^{−/−} mice) are more susceptible to colitis and GI infection, in addition to severe oral tolerance breakdown following sensitization with a food allergen. Thus, further research will be necessary to understand how functional changes in O-glycosylated mucins might contribute to the epidemic of food allergy.

Factors including reduced consumption of dietary fiber and/or increased consumption of food additives are emerging as key determinants of altered mucus barrier function and mucin–gut microbiota interactions which appear to play an important role in regulating food allergy.

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while allowing proper absorption of digested nutrients [10]. In addition to mucins, a diverse range of secreted immunological factors such as antimicrobial peptides and **secretory immunoglobulin A (sIgA)** provide potent weapons to prevent infections [14]. We discuss clinically important but unconnected links in the literature to provide clues about how the gut mucus barrier is important in maintaining **oral tolerance** and limiting exposure to immunologically active allergen residues.

An important element that regulates the GI mucosal barrier is the gut microbiome, and this has recently been proposed as an important modulator of food allergies [15–18]. Hence, the parameters that influence the mucosal barrier via gut microbial activity – such as dietary habits and maturation of the early-life immune system in the face of a developing gut microbiome – may play important roles in driving sensitization and affecting allergy onset [19–22]. As the broader aspects of the gut microbiome links in sensitization have been reviewed elsewhere [3,9,22], and evidence is mounting about how the commensal microbiota impacts the integrity and maintenance of the mucus layer [23–25], we focus our attention on the mucus–microbe interactions. We present a novel perspective on the functioning of the intestinal mucus layer, and we discuss both the beneficial and detrimental roles of mucus–microbe interactions in food allergy.

The intestinal mucus layer: a key but underappreciated component of the mucosal barrier in sensitization

O-glycosylated gel-forming mucins

Mucus layers are composed of highly O-glycosylated mucin glycoproteins that are secreted by **goblet cells (GCs)** and expand on the apical surface of the epithelium [26]. This organized layer works as a barrier to exclude gut microbes while remaining permeable to essential nutrients and macromolecules [27] (Figure 2). Moreover, the structure and composition of the mucus layer varies along the length of the digestive tract in parallel with intestinal morphology and function [27]. In the stomach, mucin-5AC (MUC5AC) is the predominant gel-forming mucin and the mucus is structured into two thick layers – a firmly adherent layer attached to the epithelium overlain by a loose adherent layer [28]. These thick layers have been proposed to work as a buffer to facilitate proton secretion while protecting the gastric tissue from the acidic luminal content [29]. In the small and large intestines, mucin-2 (MUC2) is the predominant secreted mucin, although mucus layers harbor different structures [27]. The mucus layer in the small intestine is a single loose and permeable layer that permits commensals to enter closer to epithelial cells such that these are further sampled by lamina propria antigen-presenting cells [30]. By contrast, in the colon, two distinct layers of mucus have been described, the outer layer being more loose and permeable to bacteria whereas the inner one is more firmly attached to the epithelium and is impermeable to bacteria [28,31]. Indeed, MUC2-deficient mice display a thinner and looser colonic mucus layer that permits commensals to come into close proximity to the epithelium [32]. These mice are highly susceptible to intestinal infections and have been reported to develop spontaneous colitis even in the absence of pathogens [33,34]. In addition, they show high susceptibility to the development of intestinal tumors driven by low-grade inflammation and proliferative epithelial cells, demonstrating a crucial role of the mucus barrier in preserving intestinal barrier homeostasis [35,36].

In addition to the protein backbone of mucins, O-glycosylation is an important feature that contributes to the viscoelasticity of mucus, thus promoting its activity as a lubricant to help to expel particles, parasites [37,38], and thus potential allergens. GI mucins are composed of four main types of O-glycan structural cores, in addition to terminal fucosylation, sialylation, and sulfation, and these post-translational modifications are differentially distributed along the digestive tract [11,39]. It was recently shown that differential patterning of glycosylation between the proximal and distal colon governs the formation of the two-layered structure to provide optimal exclusion of commensals and expulsion of fecal pellets [37]. O-glycosylation contributes to mucin conformation and

Glossary

Akkermansia muciniphila: a commensal bacterial member of the human and non-human gut microbiota, and a mucin-degrading specialist, that has been associated in humans with both beneficial and harmful effects in multiple disorders.

Chao diversity: the alpha diversity matrix for species richness estimates, with a focus on rare or less-abundant operational taxonomic units (OTUs).

Class-switch DNA recombination (CSR): the biological process by which B cells rearrange the immunoglobulin (Ig) heavy chain from one class to another, producing antibodies with different functions through recognition by specific Fc receptors, but without altering antigen specificity.

Congenital disorders of glycosylation (CDGs): a group of over 130 rare diseases so termed because of genetic defects in glycosylation.

Goblet cells (GC): specialized cells, located within the epithelium of mucosal surfaces including the respiratory and gastrointestinal tracts, which secrete gel-forming mucins.

Inflammatory bowel disease (IBD): immunological diseases characterized by chronic inflammation of the gastrointestinal tract; IBDs include ulcerative colitis (UC) and Crohn's disease.

Microfold (M) cells: specialized epithelial cells covering Peyer's patches in the small intestine and which can actively transport antigens from the luminal contents to the lamina propria.

Oral tolerance: an immunological process describing active unresponsiveness upon exposure to dietary antigens.

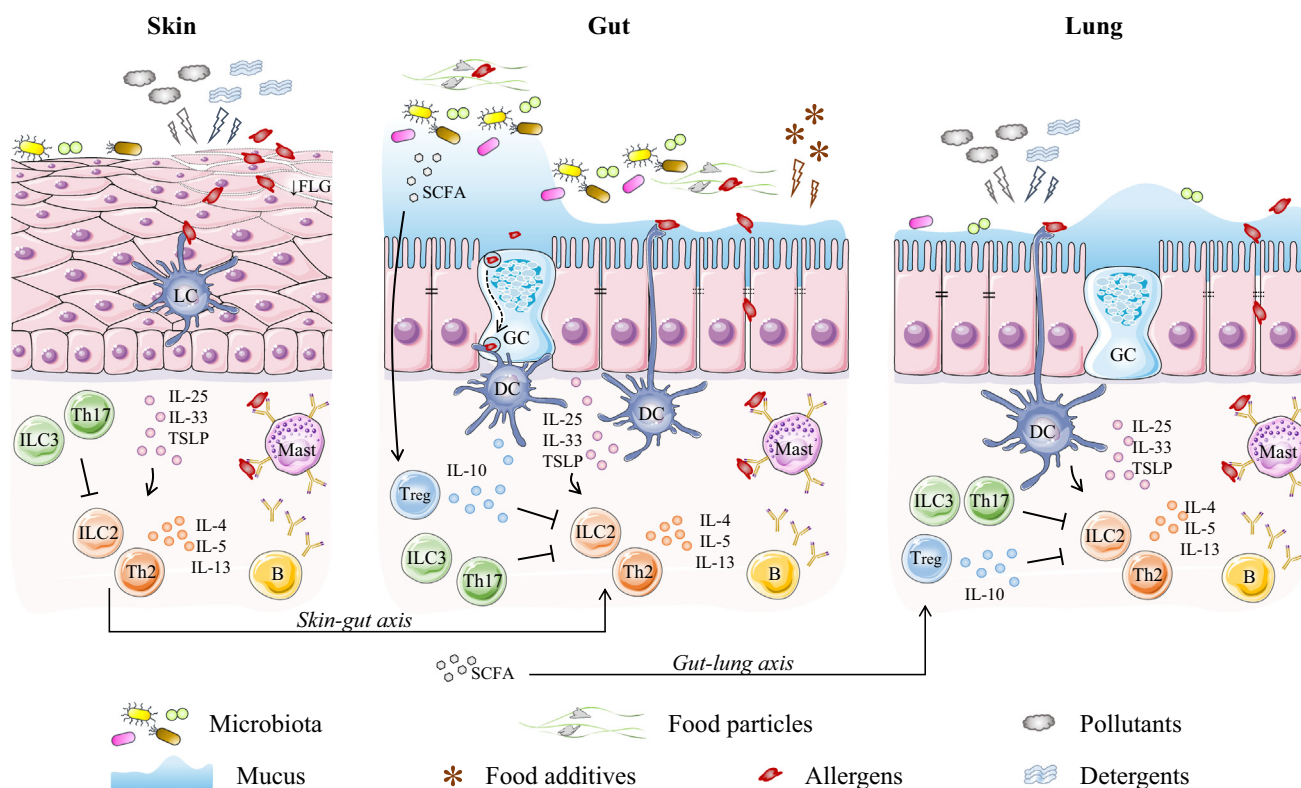
Ovalbumin (OVA): the major protein allergen found in egg white, and that is commonly used in animal experimental models for allergic sensitization.

Proctitis: inflammation of the epithelial lining of the rectum.

Secretory immunoglobulins: sIgA and sIgG are host-produced antibodies which are secreted into the luminal content and coat bacteria to limit pathogen invasiveness and enhance mucosal immune homeostasis.

Sensitization: an inappropriate immune-mediated response towards a dietary or environmental antigen which can lead to anaphylaxis.

Shannon diversity: the alpha diversity matrix which accounts for the abundance and evenness of species within an ecosystem.

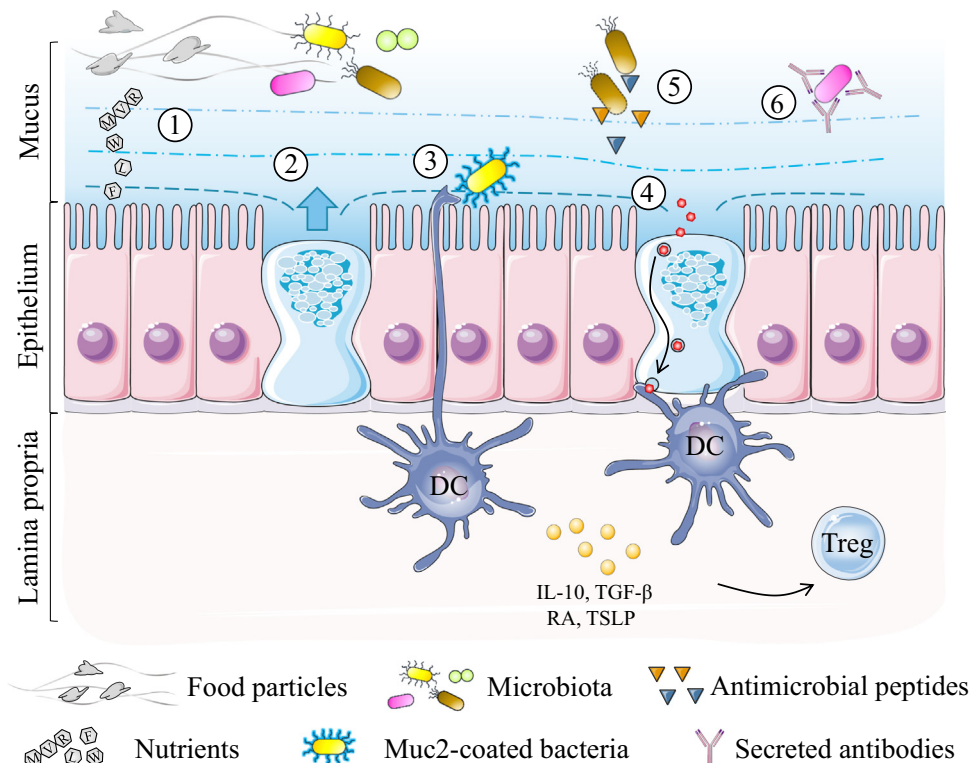


Trends in Molecular Medicine

Figure 1. Barrier dysfunction enhances allergen sensitization at multiple organ sites. As in atopic dermatitis and asthma, IgE-mediated food allergy is characterized by a strong type 2 T helper cell (Th2) immune response, IgE production and effector cell (mast cells, eosinophils, and basophils) activation, the production of type 2 cytokines such as IL-4, IL-5, and IL-13, and the production of specific IgE by B cells. Genetic susceptibilities (such as filaggrin, *FLG*), pollutants, and detergents are well-known environmental stressors that drive skin and lung epithelium leakage, which facilitates allergen uptake by local dendritic cells – Langerhans cells (LCs) in the skin and dendritic cells (DCs) in the lung – and further immune sensitization. Although epithelium leakage has been reported in food allergy, and recent studies suggest that food additives could play similar roles in the gut, barrier dysfunction is far less well described in this context. In addition, the gut is characterized by a dense microbial community that is kept at bay by a thick mucus layer secreted by goblet cells (GCs), which can also act as antigen passages (GAPs) that deliver antigens to DCs in the lamina propria. Some commensals can contribute to gut barrier homeostasis by inducing IL-22⁺ type 17 T helper (Th17) cells/type 3 innate lymphoid cells (ILC3s) and IL-10⁺ regulatory T cells (Tregs), thus promoting epithelial integrity and antimicrobial immunity, as well as preventing allergic Th2 responses. This protective immunity is fueled locally in the gut and at a distance in the lung by short-chain fatty acids (SCFA) derived from the metabolism of dietary fibers (gut–lung axis). In addition, dietary fibers prevent the expansion of mucolytic bacteria and subsequent excessive mucus foraging. It remains to be determined whether such mucus barrier dysfunction contributes to sensitization in food allergy.

plays an essential role in protecting their protein backbone from proteolysis by microbial enzymes [40,41]. Consistently, mice with defects in intestinal O-glycosylation display a disorganized and permeable colonic mucus layer and develop microbiota-dependent spontaneous colitis [40,42,43].

Recent characterization of colonic MUC2⁺ cells by single-cell RNA-seq has identified specific subpopulations of GCs that differ based on gene expression profiles, localization in the epithelium, and mucus secretion patterns [44]. At the surface of the epithelium, both crypt- and intercrypt-derived mucus generate a mucus layer that is impenetrable to bacteria-sized 1 μ m beads [44]. However, 0.2 μ m beads and 2000 kDa fluorescein-labeled dextran, mimicking macromolecules or nutrients, could only penetrate the intercrypt mucus. Intriguingly, these populations could be distinguished by differential lectin staining, revealing different glycosylation patterns of the secreted mucins [44]. This study paves the way towards a high-resolution understanding of the diverse roles of the mucus at the interface between the microbiota and the immune system.



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Figure 2. Immunoregulatory roles of the intestinal mucus layer. A healthy gut barrier maintains homeostasis with the dense microbiota through a protective mucus layer. ① The mucus layer serves as a filter to exclude food particles and commensals while remaining penetrable to essential nutrients. ② Continuous mucus secretion by goblet cells keeps luminal content away and helps to expel intestinal content in concert with intestinal motility. ③ Sensing of MUC2-coated bacteria by transepithelial dendrites and ④ goblet cell-associated antigen passages induce CD103⁺ dendritic cells (DCs) to produce IL-10, TGF- β , retinoic acid (RA), and thymic stromal lipoprotein (TSLP), thereby favoring the proliferation of regulatory T cells (Tregs). ⑤ Antimicrobial peptides and ⑥ secreted antibodies prevent bacteria from entering close contact with the epithelium and overstimulating the immune system.

Regulation of mucin production by immune mediators

Mucin production is regulated by luminal microbial products as well as by immune factors [14]. As described in the lung, type 2 inflammation elicited by parasite infections was shown to induce intestinal GC hyperplasia and mucus secretion through the IL-13–IL-4R α –STAT6 pathway [38,45]. In addition, cholinergic agonists, as well as histamine and prostaglandin E2 released by effector cells, are potent inducers of mucus secretion in the small and large intestines [46,47]. Although similar type 2 immune responses are found in food allergy, little is known about the subsequent outcome for intestinal mucus production and composition. Mucus depletion has been reported in the distal colon of food-allergic patients with **proctitis** [48]. In addition, mucus replenishment was observed after allergen avoidance, suggesting allergen-driven depletion [48]. This contrasts with allergic asthma, where IL-13-driven overproduction of mucus in the airways is a pathological outcome [49].

Colonic mucus depletion has also been described in active ulcerative colitis (UC, a type of **inflammatory bowel disease, IBD**) where sentinel GCs at the entrance of the crypts do not produce mucus in response to bacterial stimuli, in contrast to GC in healthy controls or in patients in remission [50]. Moreover, further studies have revealed that active UC patients and those in

remission exhibit decreased intercrypt GCs, leaving areas of the epithelium uncovered and exposed [44]. Studies in animal models have shown that a high demand for MUC2 synthesis leads to endoplasmic reticulum (ER) stress and GC apoptosis, which in turn leads to reduced mucin production and release [51,52]. Although high demand for MUC2 production is thought to follow mucus discharge induced by microbial stimulation or inflammation, the specific factors initiating this process remain unclear. Indeed, in contrast to type 2 T helper cell (Th2) cytokines, regulation of mucus production by Th1 and Th17 cytokines is more controversial, and further work will be necessary to understand the underlying mechanisms and level of regulation.

Although colitis (including IBD) and food allergies are etiologically distinct diseases, they often co-exist and may share similar pathological mechanisms [53–55]. Children with food protein-induced enterocolitis (FPIE), an endotype of food allergies, display low expression of the anti-inflammatory receptor TGF β RI and high expression of the proinflammatory cytokine TNF- α in the small intestinal epithelium and lamina propria [56]. TNF- α is well known to increase gut permeability and mediate colitis in IBD patients [57]. Thus, it has been proposed that increased TNF- α expression together with impaired TGF- β signal transduction could favor food antigen translocation through the gut epithelium, leading to immune sensitization [56]. Although TNF- α is considered to affect epithelial permeability principally by downregulating the expression of tight junction proteins, its impact on intestinal GCs and mucus secretion has not been studied extensively. In mouse pups, TNF- α induced mucus secretion in a TNFRI-dependent manner and *Muc2* mRNA expression in a TNFRII-dependent manner [58]; in addition, these two pathways developed differentially during the postnatal period. Cytokine-induced mucus depletion in the immature ileum emerged through TNFRI-mediated secretion, whereas TNFRII-mediated replenishment was effective only in the mature ileum [58]. This study highlights the level of complexity when studying MUC2 expression, production, and secretion, and emphasizes the need to explore mRNA as well as protein expression levels. In human mucus-secreting colonic cancer cell lines, TNF- α was reported to induce *Muc2* mRNA expression [59]. TNF- α , IL-1, and IL-6 were also reported to induce glycoprotein release from the LS160 cancer cell line [60]. Interestingly, secreted protein and carbohydrate profiles did not follow the same kinetics following proinflammatory cytokine stimulation [60], suggesting that protein expression and glycosylation patterns are differently regulated. These results are in line with data from active UC patients who were found to display shorter mucin O-glycans [61]; nevertheless, whether this glycan alteration results from inflammation or microbial metabolism remains to be determined.

In mice, *Nippostrongylus brasiliensis* and *Trichuris muris* are parasites that predominantly infect the small and large intestines, respectively, and both induce a type 2 response as well as IL-22 expression, mirroring parasite infection in human [62]. In both models, IL-22 was shown to promote worm expulsion and increase GC marker expression [62]. However, IL-22 induced *Muc2* mRNA expression in *N. brasiliensis*-infected jejunum, but not in *T. muris*-infected colon [62], suggesting that the regulatory pathways differ between organs. In different models of colitis, although IL-22 is considered to be an epithelium-protective cytokine, its role in mediating or preventing ER stress and mucus secretion remains unclear [63,64]. It was recently confirmed that IL-22 induces epithelial stem cell proliferation and glycocalyx-associated mucins at the expense of GCs and MUC2 expression [65]. Together, these studies suggest that the effect of IL-22 on GC-derived mucus production is principally environmental rather than an intrinsic and direct effect. Nevertheless, the role of IL-22 in the allergic gut remains questionable because increased plasma levels of the cytokine have been reported in infants who are allergic to cow's milk [66]. Similar findings were reported in atopic dermatitis and asthma where animal models revealed that the cytokine can have both beneficial and pathologic effects [67,68]. Further work will be necessary to determine whether colonic mucus depletion in food allergy-

associated proctocolitis is a general feature of food allergies or might define a clinical subtype of food allergy associated with a specific immunological landscape.

Role of GCs and O-glycosylated mucins in oral tolerance

Current understanding of oral tolerance is that it is orchestrated by a local regulatory T cell (Treg) response in mesenteric lymph nodes (MLNs) that is induced by luminal antigen-presenting CD103⁺ dendritic cells (DCs) from the small intestinal lamina propria (siLP) [69]. These antigen-specific FoxP3⁺ Tregs then migrate to the siLP to maintain tolerance upon re-exposure [70,71]. In addition, colonic tolerance was reported to be driven by DCs migrating to iliac lymph nodes [72]. Antigen sampling by LP DCs was initially described as an indirect process mediated by **microfold (M) cells** in gut-associated lymphoid tissues or by antigen diffusion through paracellular leaks or dendrite extensions through the epithelium [73,74]. Similar to M cells, transepithelial dendrites (TEDs) have been described in the small intestine of some (but not all) mouse strains, and these seem to be regulated by environmental stressors rather than being a homeostatic feature of the healthy intestine, questioning the existence of additional tolerogenic pathways in the colon [75]. Over the past decade, GC-associated antigen passages in the small intestine and the colon have positioned GCs as key actors in intestinal sensing and immunoregulation [76]. Indeed, following mucus exocytosis, GCs endocytose luminal antigens and transfer them to underlying antigen-presenting cells in the siLP as well as in the colonic LP [76,77]. In addition to delivering antigens, GCs were recently reported to drive oral tolerance by inducing tolerogenic DCs and macrophage-derived IL-10 production, and by retaining the LP Treg population [75].

MUC2-deficient mice display increased prevalences of Th1 and Th17 cells, and lower Treg cell counts in the si-LP [30]. Interestingly, when exposed orally to MUC2 protein from wild-type (WT) mice, deficient mice were able to shift their immune cell profiles towards increased Tregs and reduced inflammatory cell subsets, but were unable to restore the physical barrier [30]. Bacteria derived from mice as well as from human small intestine were reported to be coated with MUC2 glycoproteins [30]. Furthermore, this coating induced the production of IL-10, TGF- β , retinoic acid (RA), and TSLP by DCs and epithelial cells, and thus promoted the expansion of regulatory T cells instead of Th1 and Th17 cells (Figure 2). In addition, although TGF- β expression was microbiota-dependent, the expression of IL-10, RA, and TSLP remained lower in *Muc2*-deficient mice even after antibiotic administration, demonstrating the strong immunoregulatory properties of MUC2 proteins in the intestinal mucosa [30] (Figure 2). Importantly, when *Muc2*-deficient mice were gavaged with **ovalbumin (OVA)** before challenge with OVA, the animals were not able to develop oral immune tolerance as was observed in the WT (i.e., *Muc2*-sufficient) mice. More surprisingly, they did not develop systemic tolerance after receiving subcutaneous injections of OVA. Tolerance was, however, restored in the knockout mice when OVA was delivered in the presence of MUC2 protein, supporting a model in which the intestinal mucus layer plays a central role in preventing local as well as systemic allergy [30].

Mucin glycosylation is likely to have powerful immunoregulatory properties because epithelial and immune cells express a large panel of glycan-binding proteins or lectins [78,79]. Among them, galectin-3 has been shown to mediate MUC2-induced oral tolerance in the small intestine by activating the anti-inflammatory dectin-1–Fc γ RIIB pathway in DCs [30]. By contrast, galectin-9 expression by duodenal epithelial cells is induced by mast cell-derived tryptase and promotes intestinal type 2 inflammation and food allergy; however, the proteoglycans targeted by galectin-9 in this context have not been clearly identified [80]. Although galectins share the disacchride *N*-acetyllactosamine (LacNAc) as a common ligand, they display different affinities and

inflammatory properties depending on the glycan structure and the targeted glycosylated protein [79,81]. More recently, IL-13 was reported to induce the expression of α -1,2-fucosyltransferase 2 (FUT2) and consequently increase the abundance of fucosylation at the surface of airway epithelial cells in a model of house dust mite-induced airway hyper-reactivity [82]. This poly-fucosylation further increased airway inflammation by interacting with lectins that mediate C3 complement activation and subsequent myeloid cell recruitment [82]. In the gut, FUT2-dependent fucosylation is induced in response to type 3 innate lymphoid cell (ILC3)-derived IL-22 and lymphotoxins [83], and loss-of-function mutations in the *Fut2* gene are associated with the development of Crohn's disease [84]. Whether increased fucosylation can drive allergy in the intestine remains to be investigated. The roles of mucin glycans have been mostly studied in the context of mucus barrier integrity and colitis, and these studies argue in favor of an immunoprotective role, suggesting that a defect in these molecular patterns may also contribute to barrier breakdown and further food allergen sensitization [30].

Genes associated with pediatric food allergy were recently reviewed [85]. In addition to immune-related genes, several encode elements of the cytoskeleton and interactions with the extracellular matrix (e.g., RHOTB1, EMCN, CCDC80), proteins of the Golgi apparatus and exocytic processes (e.g., COG7, EXOC4), as well as peptidase inhibitors (e.g., SERPINB7/B2, SPINK5, ITIH5L), which may all be involved in the production, secretion, and preservation of the intestinal mucus [85]. Interestingly, mutations in component of oligomeric Golgi complex 7 (COG7) have also been associated with **congenital disorders of glycosylation (CDGs)** that affect both *N*- and *O*-glycosylation [86]. Reciprocally, patients with PMM2-CDG, a CDG that affects the *N*-glycosylation pathway, present a lower prevalence of total allergies but a higher prevalence of food allergies, as well as GI infections [87]. Similarly, mutations in the gene encoding phosphoglucomutase 3 (PGM3), that lead to decreased *N*- and *O*-glycosylation, have been linked to atopy, food allergy, and infections [88]. Although glycosylation patterns are not specific to mucin proteins, and such disorders are suspected to directly affect immune cell interactions, these studies further support a role for intestinal mucins and their glycans in food allergy.

Other components of the mucus layer

In addition to mucins, the mucus layer is composed of diverse immune factors such as host-secreted antimicrobial peptides, antibodies, and galectins which work in concert to prevent microbes and food allergens from entering close contact with the epithelium [89]. It has been reported that purified MUC2, as well as IL-1 β , ATP, and butyrate, can induce the production of the antimicrobial peptides β -defensin and cathelicidin by epithelial cells *in vitro* [90,91]. Consistently, β -defensin and cathelicidin expression was barely detectable in the colons of *Muc2*-deficient mice [90,91]. At the same time, MUC2 proteins protected pathogenic and non-pathogenic *Escherichia coli* from the antimicrobial activity of β -defensin [91]. Despite showing opposing effects, these results demonstrate that MUC2 proteins have multifaceted roles which can preserve the microbiota and prevent overactivation of the immune system by microbial debris under homeostatic conditions while supporting antimicrobial defenses in an inflammatory environment.

Immunoglobulins and IgG Fc-binding protein (FCGBP) are abundantly found in the mucus of the small and large intestines [89,92]. sIgs form immune complexes with luminal antigens and bacteria, preventing them from reaching and passing through the epithelium [93] whereas FCGBP inhibits complement activation and preserves the immunotolerant environment [92], although its IgG-binding capacity has been recently questioned [94]. Lumican is an extracellular matrix glycoprotein that is also secreted in the mucus [89]. It has been reported to facilitate the sensing of microbiota-derived lipopolysaccharide (LPS) and promote the homing of innate immune cells in the healthy intestinal mucosa, thus preventing microbiota-mediated colitis [95]. Lumican

expression can be increased in response to the proinflammatory cytokines IL-1 β and TNF- α to mediate fibrocyte differentiation and wound healing [96]. It is also overexpressed in the basement membrane of small airways from atopic asthmatic patients, and is reduced after treatment with an anti-IL5 antibody [97]. In addition to a potential role in asthma-associated airway remodeling, the function of lumican in type 2 inflammation and intestinal allergy remains unknown. Overall, the literature discussed so far highlights an important and hitherto underappreciated role of mucus in food allergy. Along these lines, the factors that affect mucus structure and integrity, including mucus–microbiome interactions in the colon, are worthy of attention.

Diet–microbiota–mucus axis in food allergy

The gut microbiota in food allergy

Several authors have identified a commensal microbiota signature associated with food allergy in children [16,98,154]. More recently, a commensal signature tied with metabolomic profile confirmed a loss of operational taxonomic units (OTUs) from the Clostridia taxon, associated with a loss in the diacylglycerol metabolism in children with food allergy compared to their healthy twins [99]. Moreover, interactions between the gut microbiota and the host mucosal immune system, as well as the role of commensal bacteria in preventing disease by promoting regulatory responses, have been extensively documented [15–17,98,100]. Notably, colonization of germ-free mice with a specific group of 46 strains of *Clostridium* spp. was able to increase the regulatory T cell population in the small and large intestines, most likely through induction of TGF- β expression by epithelial cells [17]. Thus, *Clostridium*-colonized mice showed a highly reduced OVA-specific IgE response compared to specific pathogen-free (SPF) mice [17]. In another study, *Clostridium* spp. were shown to protect the host against peanut allergen sensitization by inducing the expression of the epithelial barrier protective cytokine, IL-22, a key player that was able to block host uptake of dietary antigens [15]. In addition, the authors report an increase in GC counts in the distal colon in *Clostridium*-colonized mice [15], which suggests a potential link between the induction of mucus secretion by *Clostridium* spp. and protection against food allergy. Nevertheless, there is little knowledge about how the mucus-associated and the mucin-degrading gut microbiome impacts the pathogenesis of food allergy.

Recent data have highlighted the link between commensals and Ig coating [101–103]. IgA is the most prominent Ig found in the gut and is considered to be a crucial host defense (whether specific or non-specific) for maintaining tolerance in the gut [101]. It was recently shown that food-allergic children display an enrichment for IgE-coated bacteria together with depletion of IgA-coated bacteria [98], whereas another study reported the possibility of local IgE isotype switching in the gut of peanut-allergic patients [104]. Commensals have been described to induce the production of high-affinity specific IgA and IgG via T cell-dependent and -independent pathways [102,103]. Reciprocally, IgA was shown to regulate microbiome composition and metabolism [105–107]. However, it remains unclear what predisposes the host to undergo **class-switch DNA recombination (CSR)** from IgA or IgG to IgE in the gut [108]. Over recent years studies have demonstrated a role for the gut microbiota in driving atopic IgE hyperimmunoglobulinemia. One study reported that Clostridiales and Bacteroidales, but not Proteobacteria, could prevent the production of total and OVA-specific serum IgE in OVA-sensitized mice, in a ROR γ t⁺ Treg MyD88-dependent pathway, showing the potential of different bacterial families to protect against food allergy or mediate IgE class switching [98]. Moreover, specific IgEs directed against *Staphylococcus aureus*, a common skin commensal, have been identified in severe atopic dermatitis patients, underlining the role of host–microbe interactions at the barrier (Figure 1) [109]. It is not yet clear whether gut commensals are specific targets of high-affinity IgE or are side targets of low-affinity broad-spectrum antibodies. To this aim, elucidating the cellular and molecular pathway(s) driving IgE production is needed. In addition, although IgA and IgG have been reported to prevent

the encroachment of particular commensal species into the mucosal barrier [110], the role of secreted IgE in restraining commensals in the intestinal lumen and mucus remains to be elucidated.

Mucin-microbiota interplay modulates the microbiome composition

Although mucins are already expressed in the fetal gut, colonization by microbiota at birth boosts their production [111,112]. Similarly, germ-free mice exhibit a thin colonic mucus layer that increases after colonization [23]. Reciprocally, the nascent mucus layer serves as scaffold for the microbiota to settle [111]. Bacteria use glycans for anchoring and as a nutrient source. In this way, the mucus glycosylation profile selects the microbiota composition and, in turn, the microbiota shapes the glycosylation profile of the mucus. The selective role of the mucus in determining the microbiota composition is illustrated by *Muc2*-deficient mice which display a more diverse microbiota, as shown by increased **Shannon and Chao diversity** indices and a higher relative abundance of several genera such as *Ruminococcus*, *Eubacterium*, and *Butyrivibrio* compared to WT mice [113], suggesting that a dysfunctional mucus layer has an impact on gut microbial diversity. Similarly, mice deficient in *Fut2*, encoding α -1,2-fucosyltransferase 2 (FUT2, that is responsible for adding the terminal L-fucose to mucin glycoproteins), show a decreased relative abundance of Clostridiales and an increased relative abundance of *Bacteroides*, *Parabacteroides*, *Parasulterella*, and *Eubacterium* spp. [114], suggesting that α -1,2-fucosylation is required for colonization by Clostridiales but limits the expansion of other species. Supportive of a role for the microbiota in shaping the mucus glycosylation landscape in humans, O-glycans were found to be differentially distributed along the adult digestive tract compared to the fetal intestine [39,115]. In addition, FUT2 activity was reported to increase in the small and large intestines of weaning mice, whereas sialyltransferase activity decreased in the small intestine only, and both were diet- and microbiota-dependent [116,117]. In adult mice, antibiotic treatment reduced IL-22-induced *Fut2* expression and subsequent fucosylation [83]. Interestingly, in C-section-born infants, consumption of FUT2-fucosylated breast milk oligosaccharides was proposed to reduce the risk of developing IgE-associated allergic diseases at 2 years of age by promoting the colonization of barrier-protective Bifidobacteria [118]. Together, the previously mentioned studies support the idea that the mucus layer acts as a selective substrate for microbial colonization. The role of altered microbial taxa in the breakdown of oral tolerance in *Muc2*- and *Fut2*-deficient mice remains to be studied, noting that such breakdown has been documented in *Muc2*-deficient mice [30].

Mucosa-associated bacteria in food allergy

The host intestinal mucosal layer represents a specific niche for colonization by commensal bacterial species that are commonly considered to be mucosa-associated bacteria (MABs) [119–121]. These important microbes include bacteria such as *Lactobacillus* spp. that secrete surface adhesins to attach to host mucus and extracellular matrix components [122,123]. Moreover, MABs may be able to stimulate GC activity to excrete more mucus into the lumen, which was shown indirectly by the increase in GC counts in mice colonized with *Clostridium* spp. [15,121,124]. Given their close association with the host epithelium, these microbes may act in a beneficial way by promoting appropriate immune responses to dampen inflammation [122,125]. Thus, improper MAB colonization during early life – driven, for example, by dietary factors or overuse of antibiotics – might have implications for type 2 allergic immune responses later in life [126]. Perhaps the best-studied example of MABs are the segmented filamentous bacteria (SFBs). These have been identified in mouse and rat intestine as Gram-positive bacteria that can bind to the epithelium and induce a strong Th17 immune response [125]. SFBs primarily colonize the small intestine, where the mucus layer is thinner and looser than that in the large intestine. Although SFB have not been identified in humans, a 20-strain community derived from humans was able to induce a Th17 response by adhering to the epithelium among colonization of germ-free mice and rats [125]. As research continues to shed light on this specific

niche, functional characterization of its residents may allow us to better understand microbe–host communications. Since commensal bacteria, including MABs, have been reviewed in the context of food allergy [18,22], we focus our attention here on mucin-degrading bacteria and their potential roles in food allergy.

Mucin-degrading bacteria and their potential roles in food allergy

Mucin synthesis and degradation is a continuous and normal process [128]. Mucin-degrading bacteria (Box 1) such as *Bacteroides caccae* and *Bacteroides thetaiotamicron* also have the ability to degrade plant polysaccharides, but can increasingly turn their attention to degradation of mucin glycans in the absence of plant polysaccharides [23,129]. In the absence of fiber polysaccharides, *B. caccae* also secretes a higher amount of M60-like proteases to degrade the mucin protein backbone [23]. Thus, such mucin generalists could be more detrimental to the functional mucus barrier during the absence of dietary fibers than are mucin specialists such as *Akkermansia muciniphila*, notably because excessive degradation can become pathogenic by disrupting the first line of host defense when mucin turnover fails to keep up with mucin degradation [23]. Mucin-degrading bacteria have been shown to play a role both in altering mucosal barrier physiology and in skewing microbiome–host immune interactions during times of fiber deprivation [23,24]. In mice fed a fiber-deprived diet, excessive grazing and degradation of the mucus layer left the host with a significantly thinner mucus layer and higher susceptibility to the enteric pathogen *Citrobacter rodentium* [23,130]. In another study, fiber deprivation was shown to cause thinning as well as a more permeable and penetrable mucus layer [24]. These models of microbiome-mediated mucus erosion suggest that, like enteric pathogens [23,130] and bacterial products [131], dietary antigens could also pass through the mucus and the epithelium to stimulate an immune response. Such erosion of the intestinal mucus layer may showcase a novel mechanism that predisposes to allergic sensitization in the gut. Moreover, future studies

Box 1. Mucin-degrading bacteria

Mucin-degrading (also commonly referred as ‘mucolytic’) bacteria are members of the gut microbiome that display growth on mucins produced by their respective hosts and specific ecological colonization niches [131,140,141]. This diverse group of bacteria are commonly able to remove sialic acid [142] and/or sulfate caps [143] that render the mucin more resistant to hydrolytic attack and express glycoside hydrolases [11,141,144] and/or proteases [23,145] that degrade mucin O-glycans and/or the mucin peptide backbone, respectively.

Nevertheless, this simplified interpretation of mucin-degrading bacteria poses various practical challenges for their laboratory assignment *in silico*, *in vivo* and *ex vivo*. Several studies have considered pure cultures of bacteria that can be grown on crude porcine or human-derived mucus as mucin-degrading bacteria [131,140,141]. Other studies have used bioinformatic approaches to search for homologs of genes encoding the *Bifidobacterium bifidum* PRL2010 enzymes that are responsible for releasing mucosal sugars [146]. Although such approaches are generally informative, we need to consider that (i) crude mucus preparations may contain free mucin sugars such as fucose, galactose, sialic acid, *N*-acetylglucosamine, and *N*-acetylgalactosamine, and these may not always signify specialist mucin-degrading capacity; and (ii) the annotation of mucin-degrading carbohydrate-active enzymes, despite important work thus far [147], still has substantial ambiguity in connecting predictions to the catalytic roles of the enzymes.

Akkermansia muciniphila, *Bacteroides thetaiotamicron*, *Bacteroides caccae*, and *Barnsiella intestinihominis*, four phylogenetically distinct human gut bacterial strains, were shown to grow on purified porcine gastric mucin O-glycans [23]. It should be noted, however, that the ability of these strains to grow on purified porcine gastric mucin O-glycans is only indicative of (i) their ability to grow on colonic mucin from the same host (porcine), and potentially (ii) their ability to grow on purified mucin O-glycans from their original host (humans). This is because mucin structures differ between different mammals [12]. Although *A. muciniphila* was previously shown to be able to grow on purified porcine gastric mucin O-glycans [23], a recent study showed that *A. muciniphila* fails to grow on sulfated distal colonic mucins from the same host (porcine) [143]. This example further highlights the underlying complexity in the microbial metabolism of mucins and strongly supports metabolic interactions between various phylogenetically distinct bacteria to initiate and execute the process of mucin degradation.

need to consider the general impact of dietary additives on the microbiota–mucus axis (Box 2) and the subsequent impact on food allergy.

Interplay between beneficial, butyrate-producing, mucus-associated *Roseburia* spp. and the mucin-degrading specialist *A. muciniphila* was recently reported. Although *Roseburia* spp. cultured alone were unable to degrade porcine mucin, they were able to benefit in coculture from the sugars liberated by *A. muciniphila*, which led to an eventual increase in the amounts of the anti-inflammatory short-chain fatty acid (SCFA), butyrate [132]. Another example of cooperation is the ability of *E. coli* HS to infect the host by passing through the mucus barrier when a mucin-degrading bacterium such as *B. thetaiotamicron* cleaves the terminal sulfate from mucins [133]. This study suggests that there may be additional metabolic interactions in the intestine between the mucin-degrading and other bacteria that could be both beneficial or detrimental to the host in the context of various diseases including food allergy.

Overall, studying the impact of mucin-degrading bacteria on food allergy is an imminent area of research. *A. muciniphila* has been extensively described as being immunomodulatory, and it correlates negatively with many Western diseases including obesity and diabetes [103,134–136]. One recent study showed that the abundance of *A. muciniphila* is reduced in asthmatic patients, and that administration of *A. muciniphila* in mice significantly reduced airway hyper-reactivity and airway inflammation [136]. Consistently, impaired colonization of three mucin degraders, *A. muciniphila*, *Ruminococcus gnavus*, and *Lachnospiraceae* bacterium 2_1_58FAA, has been associated with atopic dermatitis in a subset of breast and formula mixed-fed children aged 6 months [137]. By contrast, early colonization by *R. gnavus* has been associated with increased respiratory allergy onset in infants and increased ovalbumin-induced airway hyper-responsiveness when intragastrically administered to mice [138]. *R. gnavus* administration also induced Th2 cytokine expression as well as Th2 cell and eosinophil infiltration in the mouse colonic lamina propria, suggesting potential susceptibility to food allergy [138]. More recently, *R. gnavus* was shown to be enriched in food and respiratory allergic patients' gut microbiome [154]. Further studies, especially in the context of food allergy, will be necessary to determine whether microbial mucus foraging, including the impact of specific mucin-degrading bacteria, accompanies and mediates these beneficial and detrimental effects in maintaining intestinal homeostasis and driving allergic sensitization.

Concluding remarks

We have pieced together generally unrelated aspects of independent research by different laboratories to underscore the potential role of microbiota–mucus interactions in mediating food

Clinician's corner

Food allergy prevalences are rising in Western countries, likely owing to environmental stressors that drive sensitization. Compromised barriers in the skin and lungs are known to drive allergic sensitization; however, in the gut little is known about how mucosal barrier dysfunction might lead to an allergic response.

A part of the mucosal barrier in the gut – the mucus layer – is vital for limiting exposure to dietary antigens and for maintaining oral tolerance. The colonic mucus layer is a glycoprotein-rich barrier that contains mucins and host-secreted immunological factors, such as antimicrobial peptides and IgA. The goblet cells that secrete mucin glycoproteins have recently emerged as being clinically important because they can deliver antigens to dendritic cells in the lamina propria. Nevertheless, much remains to be investigated about how various factors that drive changes in mucus secretion by goblet cells are related to sensitization.

For example, diet can modulate the gut microbiome and thereby affect the integrity of the mucus barrier. Some commensal bacteria, termed mucus-associated bacteria, colonize a specific niche within the mucus layer and may maintain a close distance to the host epithelium. These microbes have been shown to play important immunomodulatory effects. A subset of these bacteria can degrade mucins and may potentially contribute to regulating allergic sensitization.

Because lack of dietary plant fibers enhances the mucin-degrading activities of the gut microbiome, leading to barrier dysfunction and predisposing the host to colitis and infection, a similar pathogenesis mechanism for food allergy sensitization is plausible and needs to be investigated. Future research on the role of the mucus layer and mucin-degrading bacteria in the onset of food allergy could pave the way to innovative prevention and treatment strategies.

Box 2. Alteration of the microbiota–mucus axis by dietary components

One factor thought to be responsible for the increase in food allergy sensitization, as well as of various other non-communicable diseases, is the notorious 'Western diet' which is often characterized by a higher amount of fat than dietary fiber [24,63]. Similarly, high-protein diets have also been reported to affect the expression of mucus-related genes and intestinal homeostasis by altering the microbial metabolome [148,149]. Moreover, industrialization of food processing brought with it an increasing amount of chemicals, food additives, and modifiers (e. g., emulsifiers and thickeners) that are all able to dramatically alter the intestinal barrier [150,151]. Notably, the dietary emulsifiers, carboxymethylcellulose and polysorbate-80, were shown to increase bacterial penetration through the colonic mucus layer as well as intestinal permeability, thus promoting microbiota-induced colitis and metabolic syndrome in genetically susceptible mice [151]. The food thickener maltodextrin was reported to promote colonic mucus depletion and exacerbate experimental colitis in mice by inducing endoplasmic reticulum stress [152]. Finally, a high dose of saccharin, a food sweetener, or a mixture of three food additives (acesulfame potassium, benzoate, and tartrazine), was shown to break down oral tolerance when administered orally to mice together with OVA [153]. In addition to food additives, environmental pollutants such as heavy metals, pesticides, and nanoparticles have also been proposed to be mucus disruptors by affecting either mucus production by goblet cells or its degradation by commensals [150]. Overall, disruption of the mucus layer by food additives may impact the microbiota–immune system interactions and play an important role in allergic sensitization.

allergy. Although light has been shed on the potential of microbes to initiate or regulate food allergy, less is known about the factors that drive gut microbial imbalance and mediate disease pathogenesis. In this context, the mucus layer at the interface between the microbiota and the immune system may constitute a missing piece of the puzzle. Atopic diseases stem from barrier dysfunction, whether within the lung, skin, or gut (Figure 1). It is clear that the current rise of food allergy sensitization in the Western world cannot be explained by genetic factors alone, and there appears to be a strong environmental influence.

We have described a significant role for the mucus layer within the gut that acts not only as a physical barrier to luminal content, including both commensal species and dietary antigens, but also as an immunomodulatory barrier (Figure 2). Although mostly studied in the context of colitis and infections, the role and properties of the mucus layer in the allergic intestine remain largely unknown. We consider that microbiome-driven mucus barrier erosion and its immunogenic consequences are understudied factors that need to be taken into account when studying the mechanisms underlying food allergy sensitization (see Outstanding questions). When the mucus layer is disturbed, the contact between the host immune system and potential allergens increases. This may in turn lead to allergic sensitization. We propose that there is a central need to evaluate the functional role of mucus–microbe interactions and external environmental changes that could affect these interactions. Specifically, as the diet in the West shifts towards processed foods with high fat and low fiber [139], we propose the increase in intestinal barrier dysfunctions is linked to dietary factors and could be largely driven by enhanced microbial mucolytic activity. With diminished barrier protection, we hypothesize this may contribute to the sudden rise in food allergy prevalence. Further research should focus on identifying how the mucus layer can be used as a beneficial resource to control oral tolerance, and the external environmental triggers that drive mucus barrier erosion.

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Declaration of interests

The authors declare no competing interests.

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Outstanding questions

How is the composition of the intestinal mucus layer related to food allergy? Can its protein composition, glycosylation profile, thickness, and permeability define different endotypes of food allergy?

Does mucin degradation by the gut microbiota contribute to food allergen sensitization? How is this process impacted by different dietary components, including dietary fiber and food additives?

How can we target the mucus barrier to develop therapies and strategies for the prevention of food allergy?

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