Intestinal epithelial cells in tolerance and allergy to dietary antigens

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Foods consist of complex carbohydrates, proteins, and fats, which are essential for normal growth and development, energy balance, and organ metabolic health. These complex dietary components enter the gastrointestinal tract via the mouth and passage via the esophagus, stomach, and small intestine (SI) where they undergo mechanical, chemical, and proteolytic digestion to form simple fatty acids and glycerol, carbohydrates (sugars), and peptides (di- and tri-peptides and free amino acids).[1] The simple sugars and peptides are absorbed by SI intestinal epithelial cells (IECs) including enterocytes and transported via the portal circulation, whereas monoglycerides and fatty acids enter specialized lymphatic vessels known as lacteals and are transported to the liver and are circulated for the various critical functions in energy balance, growth, and development. The basic degraded food products, simple sugars, peptides, and monoglycerides, are predominantly nonimmunogenic and avoid immune recognition. However, there is clinical and experimental evidence demonstrating the presence of undigested, or partially digested, dietary proteins in the blood of healthy individuals and in different disease states (eg, food allergy), indicating the ability of immunogenic dietary proteins to pass from the gastrointestinal lumen to circulate throughout the body.

To avoid potentially harmful immunologic reactivity to these dietary proteins, the immune system has developed mechanisms of local and systemic immune unresponsiveness to dietary protein antigens termed "oral tolerance."[2] This is thought to be primarily driven by the development of dietary antigen-specific peripherally derived $CD4^+$ $FoxP3^+$ regulatory T (pTreg) cells, which are fortified by additional supportive regulatory mechanisms including $MHCII^+$ $CX3CR1^{Hi}$ IL-10–producing macrophages, protective commensal microbes, and their metabolites such as short-chain fatty acids (eg, acetate, propionate, and butyrate).[2] In food-allergic individuals, these tolerogenic mechanisms are thought to be dysregulated, leading to the production of the gastrointestinal epithelial-derived pro–type 2 cytokines IL-25, IL-33, and thymic stromal lymphopoietin. These cytokines stimulate a dietary antigen-specific $CD4^+$ $IL\text{-}4^+$ T_H2 response that promotes local antigen-specific B-cell class switch to IgE and generation of food allergen-specific IgE antibodies, amplification and activation of $CD4^+$ T_H9 cells, type 2 innate lymphoid cell–derived cytokines (IL-5 and IL-13), and mucosal mast cell IL-9–producing cells. The type-2 cytokine storm supports the expansion of the allergic effector cells, basophils, and mast cells, priming for an IgE-mediated reaction upon subsequent food allergen exposure.[2]

The anatomical site of innocuous dietary antigens uptake and how tolerance to these dietary antigens is lost, or fail to become established, and replaced by T_H2 responses, are central questions to understand and potentially treat and prevent food allergies. Recently, there has been emerging evidence from experimental-based studies demonstrating an important role for IECs in facilitating dietary antigen sampling and directing the tolerogenic versus allergic fate decision processes and dietary antigen reactions. Herein, we will review and discuss the role of IECs in dietary antigens passage across the gastrointestinal epithelial surfaces, the tolerogenic versus allergic decision fate processes, and the functionally active role the intestinal epithelium plays in food sensitization and induction of food-allergic reactions.

INTESTINAL EPITHELIA

The single-cell layer epithelium lining the gastrointestinal tract acts as a physical barrier between the gut lumen, which contains food, bacteria, fungi, viruses, environmental particulates, carcinogens, and toxins, and the inner milieu of the body and acts as a selective filter permitting the absorption of critical dietary proteins, carbohydrates, nutrients, and electrolytes, which are essential for cellular growth and survival. To serve these critical functions, the intestinal epithelium consists of a functionally diverse array of epithelial types (ie, enterocytes, goblet cells, neuroendocrine cells, tuft cells, Paneth cells, and microfold [M] cells) that promote the development and maintenance of chemical and physical barriers that simultaneously support nutrient absorption and host defense.[3,4]

To maintain the selectively permeable intestinal epithelial barrier, IECs form both a physical barrier and a chemical barrier. The physical barrier consists of adhesive complexes linking adjacent epithelial cells through complex intercellular protein networks (adhesive complexes: desmosomes, adherens junctions, and tight junctions) that seal the intercellular space between epithelial cells, limiting the passage of luminal content and exposure of the inner milieu of the body. IECs also promote the

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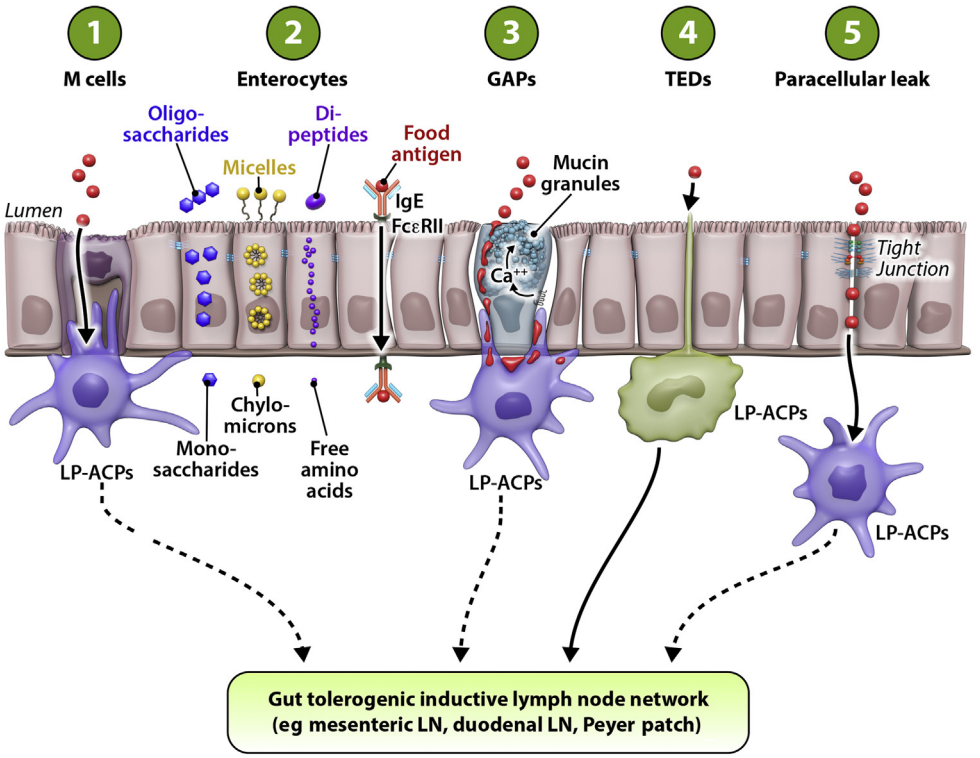

FIG 1. Dietary substances cross the epithelial barrier by intestinal epithelial–dependent and –independent mechanisms. Intestinal epithelium can directly transport dietary substances via (1) M cells, (2) enterocytes, and (3) GAPs. M cells transport GI luminal antigens to underlying immune cells via clathrin-coated endocytic and pinocytic mechanisms. Enterocytes participate in digestion via concentrating digestive enzymes at their apical surfaces that engage in lipid, carbohydrate, and protein chemical digestion. The digested nutrients are subsequently transported through the apical brush border via distinct transporters. Enterocytes can also participate in the rapid translocation of luminal allergens across the SI epithelium via low-affinity IgE receptor, FcεRII (CD23)-dependent processes. Goblet cells can form GAPs to take up and transfer large macromolecules to underlying LP-APCs. Dietary substances can cross the epithelial barrier via epithelial-independent mechanisms including (4) the extension of TEDs by LP-APCs and (5) paracellular leak between IECs through dysregulated apical tight junction complexes. Under steady-state conditions, dietary substances traversing the epithelium via transcytosis by enterocytes and paracellular leak would not be predicted to be large enough to act as antigens generating antigen-specific T-cell responses. *GI*, Gastrointestinal; *LN*, lymph node; *LP-APC*, lamina propria–antigen-presenting cells; *TED*, transepithelial dendrite.

formation of an outer chemical barrier through secretion of an array of mucin glycoproteins and antimicrobial peptides into the gut lumen, which cloaks the intestinal epithelial cell layer and limits the ability of microbes and pathogens from coming in close proximity to, or in direct contact with, the intestinal epithelia. Goblet cells (GCs), the most abundant secretory intestinal epithelial type, are the primary contributor to the chemical barrier through secretion of high-molecular-weight glycoprotein complexes, or mucus, predominantly consisting of the gel-forming mucin 2, and mucus layer–modifying proteins (trefoil factors) that form net-like polymeric sheets protecting the apical epithelial surface.[3,4] Other secretory IEC lineages include Paneth cells and enteroendocrine cells, which respectively secrete antimicrobial peptides to limit pathogen and commensal invasion and secrete hormones, such as cholecystokinin, somatostatin, serotonin, and secretin, in response to food and nutritional intake to aid with the secretion of enzymes and digestion.[3,4]

Enterocytes are the dominant IEC cell type and are primarily involved in active absorption of dietary nutrients. Enterocytes concentrate digestive enzymes (pancreatic proteolytic enzymes) at their apical surfaces, which engage in lipid, carbohydrate, and protein chemical digestion and take up these digested nutrients via apical brush border transporters (eg, SLC1A, SLC6A, and SLC7A family).[4] The other IEC populations are predominantly involved in immune surveillance. M cells are specialized absorptive cells predominantly localized to the follicle-associated epithelium covering the Peyer patches, cecal patches, and isolated lymphoid follicles. M cells transcytose luminal substances and deliver them the underlying adaptive immune compartment.[3,4] Tuft cells act as taste-chemosensory cells that can detect the presence of intestinal parasites via helminth metabolites (eg, succinate) and stimulate type 2 immune circuits that drive intestinal remodeling and helminth clearance.[4]

The basic degraded food products, simple sugars, peptides, and monoglyerides, that are absorbed by active transport processes or cross the SI epithelial barrier via paracellular leak are generally too small to act as antigens to induce antigen-specific $CD4^+$ T-cell responses. Although barrier breach due to enteric infection or diseases such as inflammatory bowel disease is associated with antigen-specific inflammatory responses to luminal substances, there is increasing evidence that IECs deliver macromolecular substances, including complex dietary antigens, from

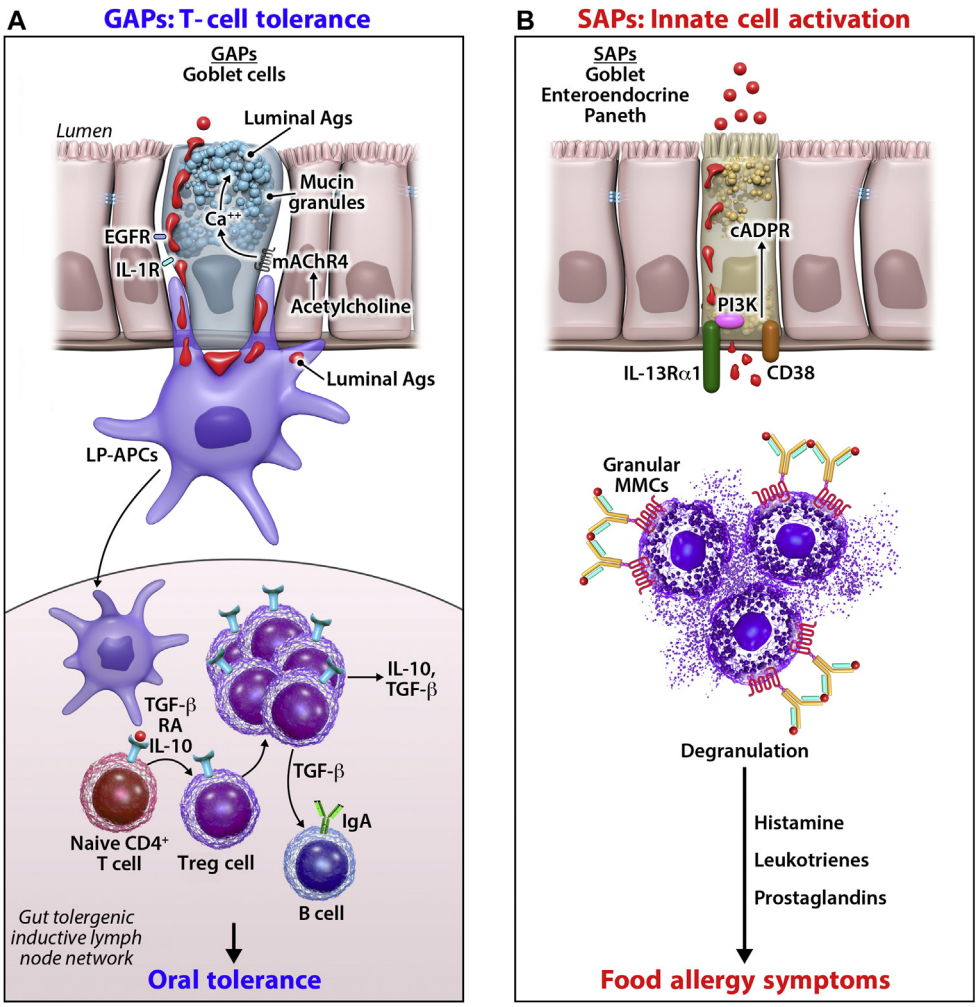

FIG 2. SI epithelial antigen passages. **A,** GAPs are present in the SI villous and crypt epithelium, induced by acetylcholine via mAChR4, and are regulated by activation of the EGFR, which can occur directly or via transactivation from the IL-1R or TLRs expressed by goblet cells. LP-APCs capture dietary antigens at the basolateral membrane of GCs and migrate via the lymphatics to drive tolerogenic responses in the draining lymph nodes. **B,** SAPs consist of GCs, enteroendocrine, and Paneth cells, and are positioned along the villus axis and crypt, regulated by the IL-13Rα1-PI3K-CD38/cADPR pathway. Mucosal mast cells capture dietary antigens at the basolateral membrane of SAPs inducing FcεRI-dependent degranulation and release of preformed mediators (eg, histamine, leukotrienes, and prostaglandins), which promote the food-allergic symptoms. *EGFR*, Epidermal growth factor receptor; *LP-APC*, lamina propria–antigen-presenting cell; *mAChR4*, muscarinic acetylcholine receptor 4; *MMC*, mucosal mast cell; *SAP*, secretory epithelial cell antigen passage.

the lumen to the underlying immune system and regulate the tolerogenic versus allergic decision fate processes (Fig 1). M cells were first identified as the IEC population involved in dietary antigen uptake and induction of tolerogenic $CD4^+$ pTreg-cell responses and oral tolerance.[5] However, disruption or absence of Peyer patches and disruption of M-cell–facilitated antigen transport to Peyer patches did not impact oral tolerance, suggesting that M cells are dispensible for oral tolerance.[5] Consistent with these observations, mice that are deficient in M cells, such as receptor activator of nuclear factor kappa-B ligand (RANKL)-deficient mice and mice with conditional deletion of TRAF6 in the intestinal epithelium, have not been associated with loss of oral tolerance. Further work revealed that enterocytes can also transcytose macromolecular substances bound to immunoglobulins to induce immune responses via enterocyte expression of the low-affinity IgE receptor, FcεRII (CD23), and the neonatal Fc receptor (FcRn). Notably, the IgE/CD23 pathway involvement in dietary allergen translocation is conserved in human IECs, and delivery of a food allergen via FcRn promotes tolerance, indicating that receptor-mediated transcytosis by enterocytes could play a role in the tolerogenic versus allergic decision fate responses to some food allergens.[6] However, FcRn deficiency does not lead to failure to thrive and food sensitization, suggesting that although FcRn can participate in these processes, there are redundant pathways delivering antigens for the induction of oral tolerance.

GCs have also recently been shown to actively participate in the uptake and translocation of luminal dietary antigens across the intestinal epithelium and deliver these antigens to the immune compartment through the formation of goblet cell-associated

antigen passages (GAPs) (Fig 2). GAPs are present in the SI and distal colon at steady state,[7] and are spatially and temporally regulated by acetylcholine via the muscarinic acetylcholine receptors expressed by GCs, and epidermal growth factor via epidermal growth factor receptor expressed by GCs and environmental factors (pathogens and commensal microbiota).[7,8] The healthy adult gut microbiota plays a critical role in suppressing GAP formation in the proximal colon. Dysbiosis of the gut microbiota allows proximal colonic GAPs to form and facilitate the translocation of commensal bacteria, providing a potential explanation for how microbiota dysbiosis can promote gut leak and alter natural oral tolerance mechanisms and promote food sensitization. Substances delivered via GAPs can be acquired by the major populations of lamina propria–antigen-presenting cells and macrophages to drive antigen-specific T-cell responses. We speculate that the tightly regulated temporal and regional control of GAPs allows luminal antigens to be delivered to discrete immunologic niches, driving immune education and tolerogenic responses to dietary and microbial antigens while limiting the immune system's exposure to more toxic substances and potential pathogens. We have previously demonstrated that dietary antigens such as ovalbumin and food allergens (peanut, egg, and cow's milk) can be delivered via GAPs and that GAPs are present in healthy human jejunal resection specimens as well as human intestinal organoids, which have the capacity to acquire food allergens such as cow's milk.[9] Thus, GAPs likely play a significant role in immune responses to food allergens in humans.

We recently examined the importance of GAPs in the regulation of the tolerogenic versus allergic decision fate outcomes for dietary antigens in mice. In adult (postweaning) mice, GAPs are present throughout the SI and a few GAPs are present in the very distal colon (distal descending colon and sigmoid colon). GAPs in the SI of weaned mice support oral tolerance through the induction and maintenance of pTreg cells and by imprinting lamina propria–antigen-presenting cells with tolerogenic properties. This observation aligns with recent studies identifying regional differences in immune responses to luminal substances in the gastrointestinal tract of adult mice. Furthermore, we identified that during a defined preweaning period, GAPs are absent from the SI but present in the proximal colon. Colonic GAPs forming during this preweaning phase support the induction of tolerance to luminal substances, leading to the development of longer-lived population of antigen-specific pTreg cells.[10] These events were under maternal control via chronological changes in breast milk epidermal growth factor. Desynchronization of mothers and offspring through asynchronous cross-fostering resulted in alterations of loss of colonic GAPs and subsequent failure to induce these longer-lived pTreg cells. As a consequence, mice became T_H2 skewed and oral tolerance to newly encountered dietary antigens was impaired.[10] These experimental analyses suggest that GAPs may play an important role in dietary antigen tolerogenic versus allergic decision fate outcomes.

In a sensitized food-allergic individual, oral exposure to the eliciting food can lead to cross-linking of the food allergen–IgE-FcεR complex on mast cells and basophils and secretion of autacoid mediators that act on target organs (gastrointestinal, cutaneous, respiratory, and cardiovascular organs) and incite the clinical manifestations of disease. We recently examined the contribution of IECs to deliver food allergens in the secondary effector phase reaction of food allergy. We found that in food-allergic mice, dietary antigens are rapidly taken up by SI GAPs and delivered to underlying IgE-bound mucosal mast cells, leading to mast cell degranulation and induction of the food-allergic symptoms[9] (Fig 2). Interestingly, in food-allergic mice, in addition to GCs, enteroendocrine and Paneth cells participated in antigen uptake, which we collectively refer to as intestinal secretory epithelial cell antigen passages (SAPs). Interestingly, the secretory epithelial cell antigen passages were not responsive to m4AchR signaling but predominantly regulated by the T_H2 cytokine IL-13 via a direct IL-4Rα- signal transducer and activator of transcription 6 (STAT6)–independent PI3K-CD38-cADPR–dependent process and necessary for effector phase of food-allergic reactions.[9] Collectively, these studies suggest that the antigen passage landscape within the gastrointestinal compartment of food-allergic mice is dysregulated, leading to additional mechanisms and IECs that contribute to antigen uptake and suggest that dietary antigens can be redirected from lamina propria–antigen-presenting cells to mast cells.

Although experimental and clinical studies support a role for skin exposure in dietary antigen sensitization and initiation of the $CD4^+$ T_H2-sensitizing response and food allergy, there is mounting evidence that IECs deliver dietary allergens to the immune compartment for the induction of immune responses and for IgE-mast cell–mediated reactions. Delineation of the IEC:dietary protein antigen axis in tolerogenic and food-sensitized states and the interaction between cutaneous- and gastrointestinal-antigen–induced responses to dietary antigens in the immunologic outcomes to foods will be critical in our understanding of the mechanisms of food sensitization and food-allergic responses.

REFERENCES

1. Said HM. Physiology of the gastrointestinal tract, 6th ed. London, UK: Academic press; 2012.
2. Yu W, Freeland DMH, Nadeau KC. Food allergy: immune mechanisms, diagnosis and immunotherapy. Nat Rev Immunol 2016;16:751-65.
3. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol 2014;14:141-53.
4. Allaire JM, Crowley SM, Law HT, Chang SY, Ko HJ, Vallance BA. The intestinal epithelium: central coordinator of mucosal immunity. Trends Immunol 2018;39:677-96.
5. Pabst O, Mowat AM. Oral tolerance to food protein. Mucosal Immunol 2012;5:232-9.
6. Ohsaki A, Venturelli N, Buccigrosso TM, Osganian SK, Lee J, Blumberg RS, et al. Maternal IgG immune complexes induce food allergen-specific tolerance in offspring. J Exp Med 2018;215:91-113.
7. Knoop KA, Newberry RD. Goblet cells: multifaceted players in immunity at mucosal surfaces. Mucosal Immunol 2018;11:1551-7.
8. Kulkarni DH, Newberry RD. Intestinal macromolecular transport supporting adaptive immunity. Cell Mol Gastroenterol Hepatol 2019;7:729-37.
9. Noah TK, Knoop KA, McDonald KG, Gustafsson JK, Waggoner L, Vanoni S, et al. IL-13-induced intestinal secretory epithelial cell antigen passages are required for IgE-mediated food-induced anaphylaxis. J Allergy Clin Immunol 2019;144:1058-73.e3.
10. Knoop KA, McDonald KG, Coughlin PE, Kulkarni DH, Gustafsson JK, Rusconi B, et al. Synchronization of mothers and offspring promotes tolerance and limits allergy. JCI Insight 2020;5:e137943.