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Rodney D. Newberry, MD, Simon P. Hogan, PhD

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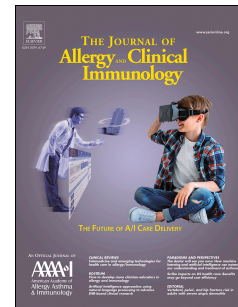
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1 **Intestinal epithelial cells in tolerance and allergy to dietary antigens**

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3 Rodney D. Newberry, MD^a and Simon P. Hogan, PhD^b

4 ^aDepartment of Internal Medicine, Washington University School of Medicine, St. Louis,
5 Missouri, USA, 63110

6 ^bMary H. Weiser Food Allergy Center, Department of Pathology, University of Michigan
7 School of Medicine, Ann Arbor, MI 48109

8
9
10 *Correspondence to: rnewberry@wustl.edu or sihogan@med.umich.edu

11
12 Simon Hogan, PhD
13 University of Michigan Medical School
14 Pathology
15 109 Zina Pitcher Pl
16 Ann Arbor, MI 48109-2200
17 734 647 9923
18 sihogan@med.umich.edu
19

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25 R.D.N. is an inventor on U.S. Nonprovisional Application Serial No. 15/880,658
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28
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Foods consist of complex carbohydrates, proteins and fats that are essential for normal growth and development, energy balance and organ metabolic health. These complex dietary components enter of 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), peptides (di- tri-peptides and free amino acids) ¹. The simple sugars and peptides are absorbed by SI intestinal epithelial cells (IEC)s 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 non-immunogenic 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 (e.g. Food Allergy) indicating the ability of immunogenic dietary proteins to pass from the gastrointestinal lumen to a circulate throughout the body.

To avoid potentially harmful immunological 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⁺ T regulatory cells (pTregs) 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 (e.g. 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 (TSLP). These cytokines stimulate a dietary antigen specific CD4⁺ IL-4⁺ Th2 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⁺ Th9 cells, innate lymphoid cell (ILC)2-derived cytokines (IL-5 and IL-13) and mucosal mast cell IL-9 producing (MMC9) 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 are lost, or fail to become established, and replaced by Th2 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 intestinal epithelial cells (IECs) in facilitating dietary antigen sampling and directing the tolerogenic vs 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 vs

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, 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 that are essential for cellular growth and survival. To serve these critical functions the intestinal epithelium consists of a functionally diverse array of epithelial types (i.e., 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 and 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 formation of an outer chemical barrier through secretion of an array of mucin glycoproteins and antimicrobial peptides into the gut lumen that cloaks the intestinal epithelial cell layer and limits the ability of microbes and pathogens from coming in close proximity or in direct contact to 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 that engage in lipid, carbohydrate, and protein chemical digestion and take up these digested nutrients via apical brush border transporters (e.g., 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's patches (PP), 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 (e.g. succinate) and stimulate Type-2 immune circuits that drive intestinal remodeling and helminth clearance⁴.

The basic degraded food products, simple sugars, peptides and monoglycerides that are absorbed by active transport processes or cross the SI epithelial barrier via paracellular leak are too small to act as antigens to induce antigen specific CD4⁺ T cell responses. While barrier breach due to enteric infection or diseases such as inflammatory bowel disease are associated with antigen specific inflammatory responses to luminal substances, there is increasing evidence that IECs deliver macromolecular substances, including complex dietary antigens, from the lumen to the underlying immune system and regulate the tolerogenic vs allergic decision fate processes (Figure 1). M cells were first identified as the IEC population involved in dietary antigen uptake and induction of tolerogenic CD4⁺ pTreg responses and oral tolerance⁵. However, disruption or absence of PP and disruption of M-cell facilitated antigen transport to PPs did not impact oral tolerance suggesting that M-cells are dispensible for oral tolerance⁵. Consistent with these observations, mice that are deficient in M-cells, such as 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 intestinal epithelial cells and delivery of a food allergen via FcRn promotes tolerance, indicating receptor mediated transcytosis by enterocytes could play a role in immune responses to some food allergens⁶. However FcRn deficiency does not lead to failure to thrive and food sensitization suggesting that while 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 antigen passages (GAPs) (Figure 2). GAPs are present in the small intestine (SI) and distal colon at steady state⁷, and are spatially and temporally regulated by acetylcholine (ACh) via the muscarinic ACh receptors expressed by GCs, and epidermal growth factor (EGF) via EGFR 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 (LP) antigen presenting cells (APCs) 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 immunological 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 (HIOs), which have the capacity to acquire food allergens such as cows milk⁹. 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 vs allergic decision fate outcomes for dietary antigens in mice. In adult (post-weaning) mice, GAPs are present throughout the small intestine 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 pTregs and by imprinting LP-APCs 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. Further we identified that during a defined pre-weaning period GAPs are absent from the small intestine but present in the proximal colon. Colonic GAPs forming during this pre-weaning phase support the induction of tolerance to luminal substances leading to the development of longer-lived population of antigen-specific pTregs¹⁰. These events were under maternal control via chronological changes in breastmilk EGF. 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 pTregs. As a consequence, mice became Th2 skewed and oral tolerance to newly encountered dietary antigens was impaired¹⁰. These experimental analyses suggest that GAPs may play an important role in dietary antigen tolerogenic vs allergic decision fate outcomes.

In a sensitized food allergic individuals, 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 symptoms⁹ (Figure 2). Interestingly in food allergic mice, in addition to GCs, enteroendocrine and Paneth cells participated in antigen uptake to some degree, which we collectively refer to as intestinal secretory epithelial cell antigen passages (SAPs). Interestingly, the SAPs were not responsive to M4AChR signaling but predominantly regulated by the Th2 cytokine IL-13 via a direct IL-4Rα-STAT6-independent PI3K-CD38-cADPR-dependent process and necessary for effector phase of food allergic

198 reactions⁹. Collectively, these studies suggest that the antigen passage landscape
199 within the gastrointestinal compartment of food allergic mice is dysregulated leading to
200 additional mechanisms and IECs that contribute to antigen uptake and may suggest that
201 in this setting dietary antigens are redirected away from LP-APCs to mast cells.

202 While experimental and clinical studies support a role for skin exposure in dietary
203 antigen sensitization and initiation of the CD4⁺ Th2 sensitizing response and food
204 allergy, there is mounting evidence that IECs deliver dietary allergens to the immune
205 compartment for the induction of immune responses and for IgE-mast cell mediated
206 reactions. Delineation of the IEC:dietary protein antigen axis in tolerogenic and food-
207 sensitized states and the interaction between cutaneous- and GI-antigen induced
208 responses to dietary antigens in the immunological outcomes to foods will be critical in
209 our understanding of the mechanisms of food sensitization and food allergic responses.

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Figure 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) goblet cell associated antigen passages (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 surface 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 lamina propria antigen presenting cells (LP-APCs). Dietary substances can cross the epithelial barrier via epithelial-independent mechanisms including 4) the extension of transepithelial dendrites (TEDs) by LP-APCs and 5) paracellular leak between intestinal epithelial cells 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.

Figure 2. SI epithelial antigen passages. A. Goblet cell antigen passages - GAPs are present in the SI villous and crypt epithelium, induced by acetylcholine via mAChR4 and are regulated by activation of the epidermal growth factor receptor (EGFR), which can occur directly or via transactivation from the IL-1R. 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. Secretory cell antigen passages- SAPs consist of GCs, enteroendocrine and Paneth cells, are positioned along the villus axis and crypt, regulated by IL-13R α 1-PI3K-CD38/cADPR pathway. Mucosal Mast cells capture dietary antigens at the basolateral membrane of SAPs inducing Fc ϵ RI-dependent degranulation and release of preformed mediators (e.g. histamine, Leukotrienes and Prostaglandins) which promote the food allergic symptoms.

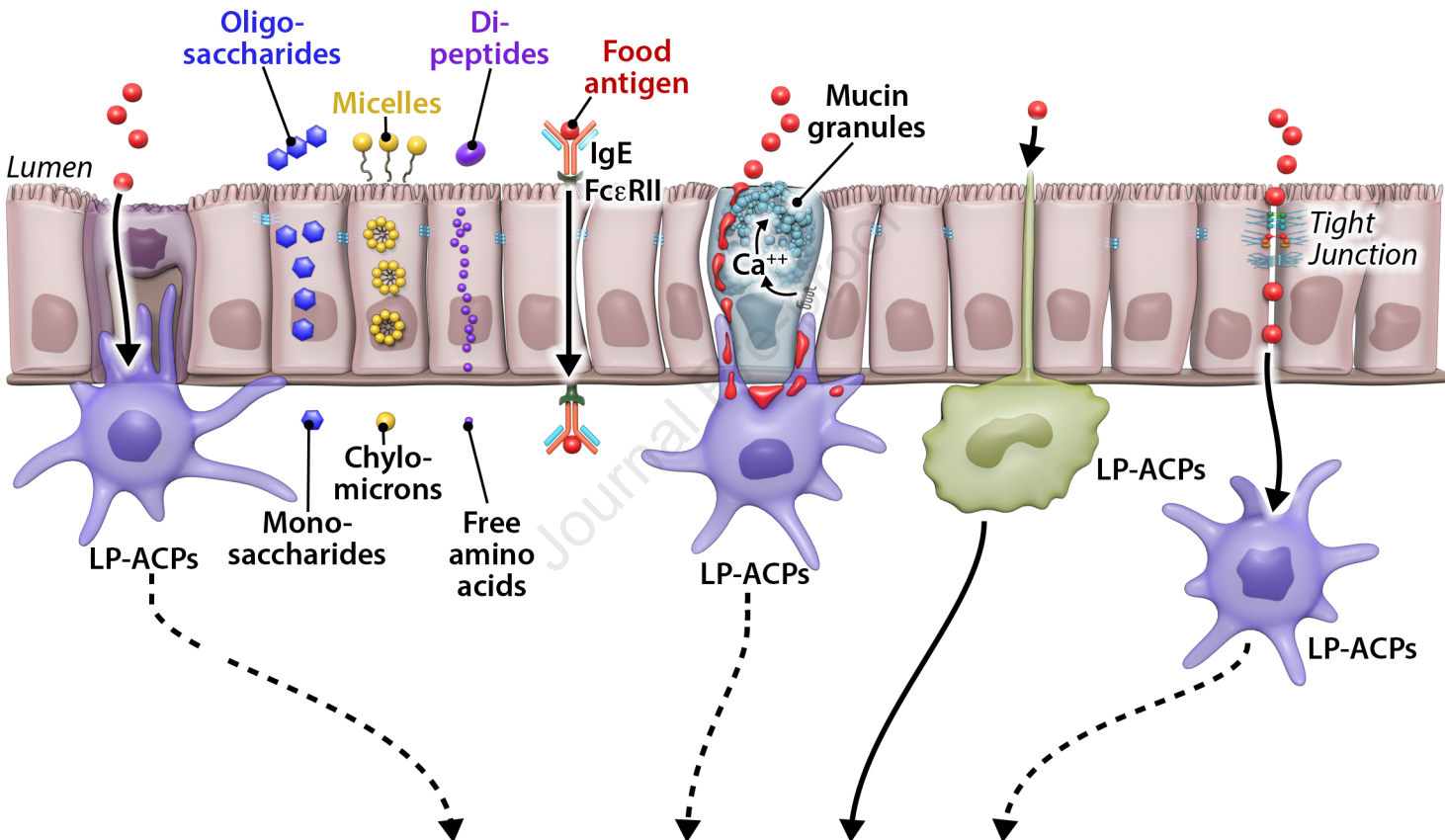


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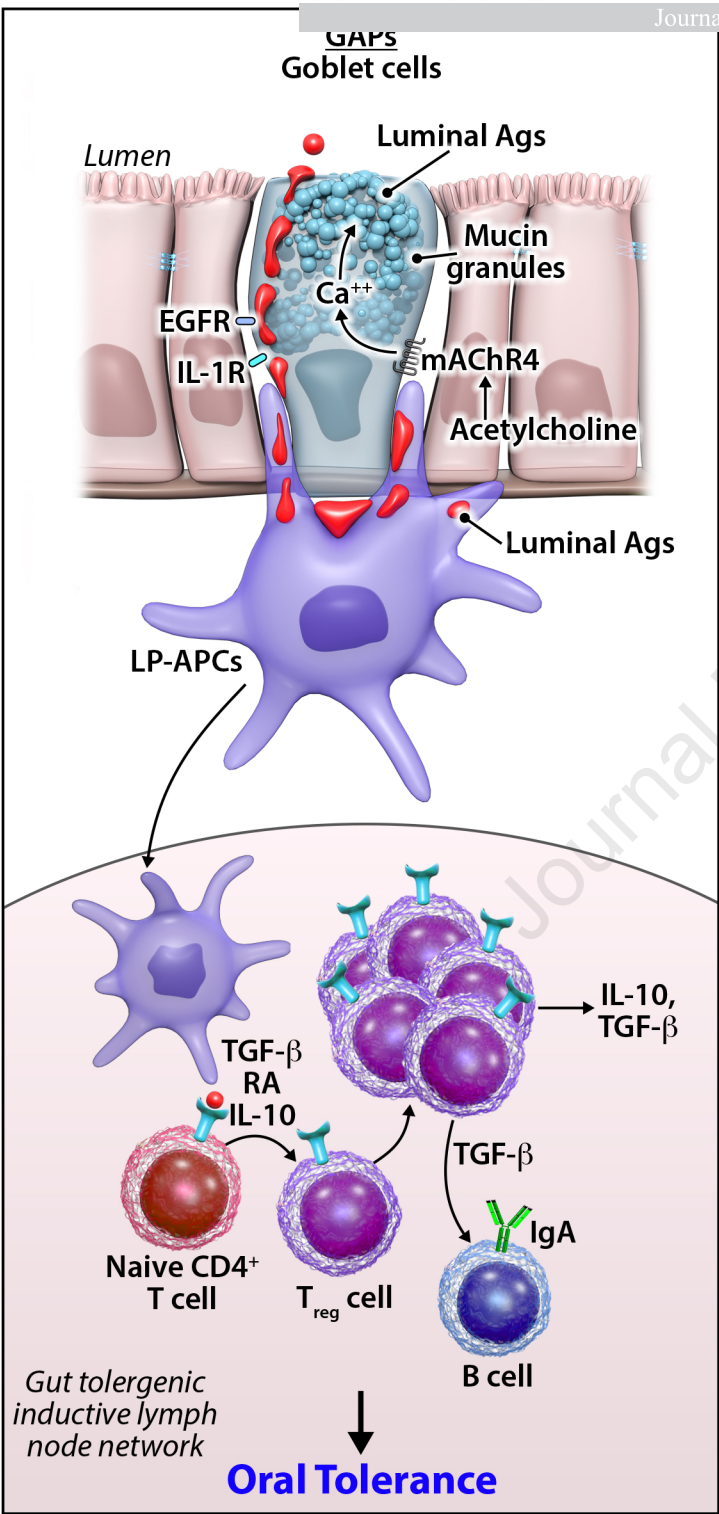
Paracellular leak



Gut tolerogenic inductive lymph node network
(e.g. mesenteric LN, duodenal LN, Peyer's patch)

A

GAPs: T cell Tolerance



B

SAPs: Innate Cell Activation

