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REVIEW

Intestinal Macromolecular Transport Supporting Adaptive Immunity

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SUMMARY

Transport of luminal antigens across the intestinal epithelium and their encounter with the underlying immune system is critical for generation of appropriate immunity. This review describes our current understanding of how macromolecules traffic the intestine and the subsequent responses.

The gastrointestinal tract performs opposing functions of nutrient absorption, barrier maintenance, and the delivery of luminal substances for the appropriate induction of tolerogenic or protective adaptive immunity. The single-layer epithelium lining the gastrointestinal tract is central to each of these functions by facilitating the uptake and processing of nutrients, providing a physical and chemical barrier to potential pathogens, and delivering macromolecular substances to the immune system to initiate adaptive immune responses. Specific transport mechanisms allow nutrient uptake and the delivery of macromolecules to the immune system while maintaining the epithelial barrier. This review examines historical observations supporting macromolecular transport by the intestinal epithelium, recent insights into the transport of luminal macromolecules to promote adaptive immunity, and how this process is regulated to promote appropriate immune responses. Understanding how luminal macromolecules are delivered to the immune system and how this is regulated may provide insight into the pathophysiology of inflammatory diseases of the gastrointestinal tract and potential preventative or therapeutic strategies. *(Cell Mol Gastroenterol Hepatol 2019;7:729–737; https://doi.org/10.1016/j.jcmgh.2019.01.003)*

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The primary function of the gastrointestinal tract is the digestion and absorption of nutrients to provide for energy and growth. This function necessitates that the lining of the intestinal tract be semipermeable and environmentally exposed. Paradoxically, because of the abundant microbes and potential pathogens within this environment, this single-layer epithelium also must act as a physical barrier to protect the host. Furthermore, it has become appreciated that the immune system underlying this epithelium is continually exposed to luminal macromolecules to allow the appropriate induction of adaptive immune responses to promote tolerance in the steady state and protective immunity during infection. How the epithelium lining the gastrointestinal tract performs these apparently opposing tasks is a central question to intestinal physiology and mucosal immunity, which may be answered in part by recent observations. This review discusses historical observations suggesting macromolecules cross the intestinal epithelium, distinctions between the pathways of nutrient absorption and pathways supporting adaptive immunity, the models of transport across the epithelium, and immune responses toward luminal macromolecules.

Historical Overview of Macromolecular Transport

Gastrointestinal physiology supports that nutrients, such as lipids, carbohydrates, and proteins, are first digested and then absorbed as small molecules in the small intestine. For example, carbohydrates are broken down into monosaccharides and transported into enterocytes by specific transporters; proteins are digested into free amino acids, dipeptides, and tripeptides; transported into enterocytes by specific transporters; and digested into amino acids for release; and lipids are digested into monoglycerides and free fatty acids, which freely diffuse into enterocytes for reassembly, packaging into chylomicrons, and extrusion on the basolateral surface. Proteolytic enzymes, serum proteins, glycoproteins, lipids, and mucopolysaccharides contribute to the array of macromolecules in the gut lumen. Studies on gastric secretions and duodenal fluids have indicated that proteins and molecules with appreciable protein content constitute the major fraction (approximately 60%) of nondialyzable substances in the gastrointestinal tract. The remaining 25%–30% is mainly carbohydrates with small but undetermined amounts of lipid and nucleic acids.[1,2] In addition, the gut microbial community contributes to the luminal contents directly

Abbreviations used in this paper: **APC, antigen-presenting cell; CX_3CR1, C-X3-C Motif Chemokine Receptor 1; DC, dendritic cell; EGFR, epidermal growth factor receptor; Foxp3+, forkhead box P3; GAP, goblet cell–associated antigen passage; IL, interleukin; LP, lamina propria; M cell, microfold cell; MLN, mesenteric lymph node; PP, Peyer's patch; SI, small intestine; TED, transepithelial dendrite; TLR, Toll-like receptor; Treg, regulatory T cell.**

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Table 1. Intestinal Microorganisms and Products Contributing to Host Immunologic, Metabolic, and Physiological Processes

Product	Microbes involved	Function	References
Short-chain fatty acids (acetate, propionate, and butyrate)	*Clostridium*, *Bacteroides*	Energy source, immune cell regulation, epithelial barrier integrity	72–75
Intermediate fermentation products (succinate/lactate)	Lactic acid bacteria, *Bifidobacteria*	Contribute to final pool of short-chain fatty acids	76,77
Microbial biotransformation of bile acids	*Bacteroides*, *Bifidobacterium*, *Lactobacillus*	Metabolic processes	78–80
Vitamin synthesis (vitamin K, biotin, folates, riboflavin, and so forth)	*Firmicutes*, *Actinobacteria*, *Fusobacteria*	Metabolic and physiological significance	81–83
Polysaccharide A	*Bacteroides fragilis*	Immune modulator	84–87

through the production of microbial products and metabolites (Table 1).

Although digestion and absorption of small molecules accounts for the vast majority of dietary substances crossing the epithelium, that this would be the only way in which dietary substances traverse the epithelium is at odds with the growing understanding that the immune system underlying the epithelium actively samples the luminal contents throughout life to induce antigen-specific tolerance to dietary substances in the steady state. Antigen-specific tolerance occurs when intestinal dendritic cells (DCs) acquire luminal substances and migrate to the mesenteric lymph nodes (MLNs) to stimulate antigen-specific naive T cells to promote the generation of forkhead box P3 (Foxp3+)-induced regulatory T cells (Tregs).[3] These newly committed induced Tregs (iTregs) migrate back to the intestine where they undergo secondary expansion by macrophages producing interleukin (IL)10[4] to suppress inflammatory responses to innocuous substances from the gut lumen. The differentiation of Tregs from naive T cells requires stimulation by DCs containing major histocompatibility complex II loaded with cognate antigen. Typically, DCs acquire proteins and process them into peptides for T-cell stimulation. The peptides loaded onto major histocompatibility complexes are significantly larger than the free amino acids released by enterocytes after digestion.[5] Thus, pathways to deliver luminal macromolecules to DCs underlying the epithelium to induce adaptive immunity must exist, however, the pathways of macromolecular transport supporting this process have been controversial.

The properties of the luminal substances that are taken up may have an impact on the subsequent immune responses. Food hypersensitivity is related to the failure of oral tolerance, and uptake of intact antigens untreated with digestive enzymes have been shown to promote allergy. Moreover, intestinal administration of intact bovine serum albumin has been shown to enhance anti–bovine serum albumin responses.[6,7] Indeed, the inability to digest a 33mer gliadin peptide containing multiple immunodominant epitopes is a central contributor to celiac disease pathogenesis.[8] Thus, digestion is a crucial process for nutrition, but also for maintaining appropriate immune responses.

In contrast to the earlier-described model of nutrient digestion and absorption, more than 50 years ago studies identified that macromolecular substances up to the micron range could be found in the blood after oral administration of compounds to animals and humans.[9] This phenomenon was termed *persorption*, because it was believed to be distinct from the process of nutrient absorption described earlier.[10,11] The absorbed particles were found in many body fluids, such as blood, lymph, bile, milk, and urine, and early speculation that the passage of particulate matter across the intestinal barrier arose from activity of the muscularis mucosa layer potentially squeezing particulates into epithelial cells.[10,11] Although persorption has long been observed, it has remained a relatively understudied area and accordingly the mechanistic details and the physiologic role of persorption remain enigmatic, with some studies implying persorption of food substances may contribute to disease.[12–14]

Pathways to Cross the Intestinal Epithelium

Multiple pathways by which luminal substances cross the intestinal epithelial barrier have been identified (Figure 1). Not shown are an array of proteins facilitating the transport of small molecules including anions, cations, zwitterions, lipids, small-molecule drugs, and small proteins.[15] Although these transporters are significant contributors to gastrointestinal physiology, the molecules they transport are generally too small to be targets for antigen-specific T-cell responses, and are not discussed further here. Conceptually, the simplest pathways delivering large macromolecules across the epithelium is barrier disruption resulting from the loss of epithelial integrity, allowing luminal substances to come into direct contact with underlying immune cells. This occurs during disease processes such as inflammatory bowel disease, in which large portions of the epithelial surface are ulcerated. However, the contribution of barrier disruption to the delivery of luminal substances to the immune system in the healthy intestine is less likely. Moreover, barrier disruption is an uncontrolled process, which would be relatively incompatible with regulating immune responses to luminal substances in the steady state. Indeed, in the absence of disease processes, the translocation of whole live bacteria can occur in the absence of barrier disruption,[16–18] suggesting the existence of other

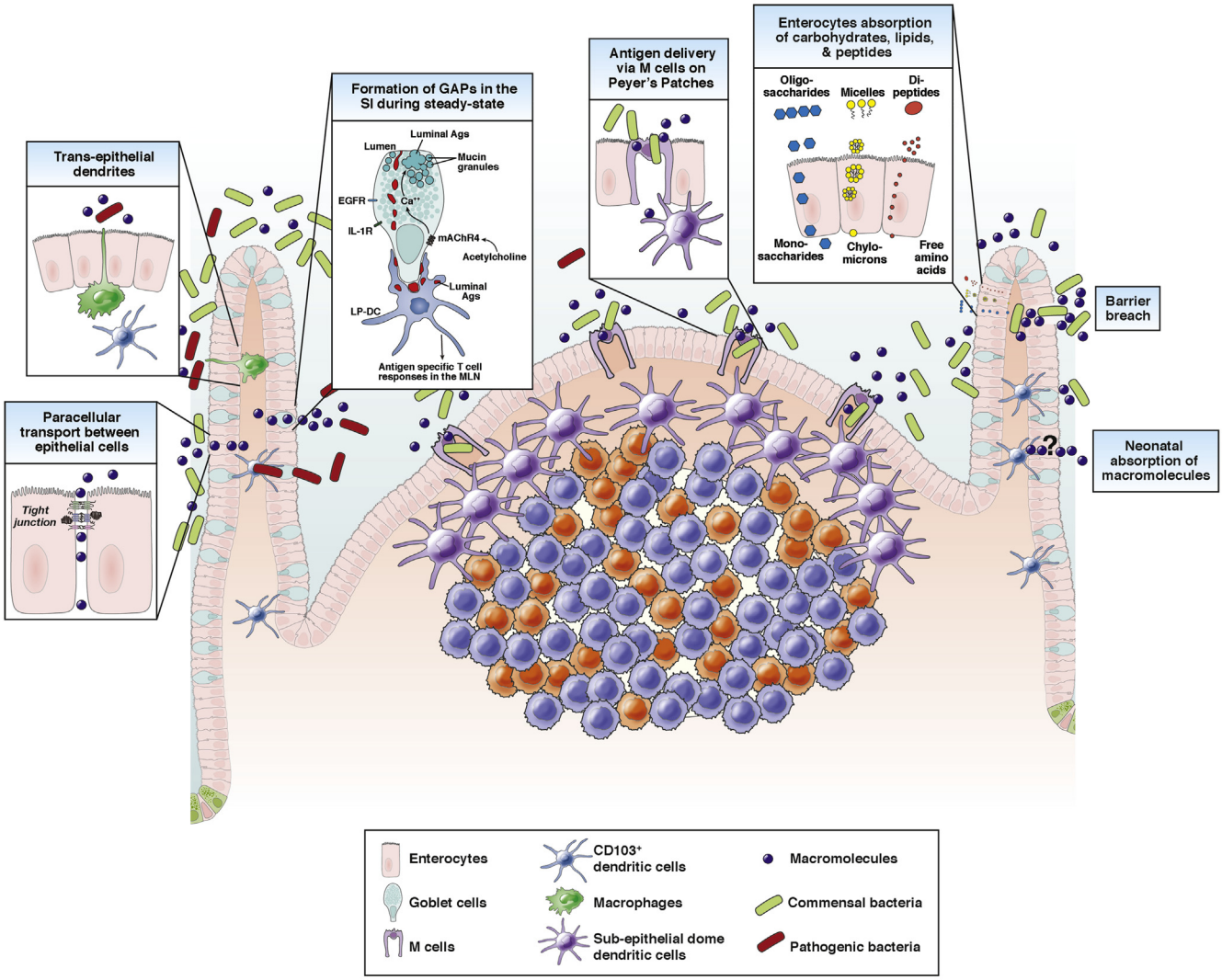

Figure 1. Pathways of specific transport mechanisms allow nutrient uptake and the delivery of macromolecules to the immune system while maintaining the epithelial barrier. Low-molecular-weight substances leak through tight junctions between enterocytes in paracellular leaks. Tissue resident macrophages can extend dendrites into the lumen and capture luminal antigens. Intestinal goblet cells take up luminal antigens and deliver them to antigen-presenting cells in the lamina propria via GAPs. M cells are specialized transcytotic cells that are located on the follicle-associated epithelium overlying Peyer's patches that transport soluble antigens and bacteria to antigen-presenting cells in the follicles. Nutrient uptake involves enterocyte absorption of smaller molecules such as monosaccharides, lipids, and dipeptides.

pathways and the lack of need to grossly compromise the epithelial barrier to deliver luminal substances of significant size.

Paracellular transport involves the passage of luminal substances through tight junctions that form the major barrier between epithelial cells. At least 2 pathways of paracellular transport through tight junctions exist: a high-capacity pore allowing for the passage of small ions and uncharged particles, and a low-capacity leak pathway allowing for the passage of larger molecules.[19] Both the pore and leak pathway are dynamic and can be regulated by inflammatory stimuli,[20] with the pore pathway's capacity being defined by the number of pore-forming claudins expressed in the tight junction and the leak pathway's capacity being associated with the number of tight junction strands present between the epithelial cells. Although these pathways are well studied and could contribute to the delivery of substances to DCs underlying the epithelium, in general the size of the substances traversing tight junctions and pores in the healthy state would not be sufficient for the induction of antigen-specific T-cell responses that are characteristic of tolerance in the steady state.

Microfold cells (M cells) were first described in the follicle-associated epithelium overlying Peyer's patches (PPs) and lymphoid follicles in the intestine.[21] These cells had unique morphology including a lack of microvilli, a rigid brush border, and a reduced glycocalyx. M cells have an apical surface with membrane microdomains facilitating endocytosis of particulates from the lumen. The basolateral surface of M cells is deeply invaginated to allow for a pocket

containing lymphocytes and antigen-presenting cells (APCs). This arrangement allows for the basolateral surface to come within microns of the apical surface, significantly reducing the distance that luminal substances would travel during transcytosis. Indeed, M-cell–mediated transcytosis is the most studied pathway delivering luminal substances across the epithelium for the induction of adaptive immune responses. Most studies support that M cells function to transport luminal substances to induce IgA and T-cell responses in the PPs and lymphoid follicles. Some studies have shown induction of tolerance by targeting antigens directly to M cells,[22] suggesting that M cells overlying PPs are a pathway supporting steady-state, antigen-specific, T-cell responses. In addition, enrichment of antigen-specific Tregs have been found in PPs of orally tolerized mice.[23] CD4 T cells expressing transforming growth factor-β II receptor are enriched in PPs of C57BL/6 mice after high- or low-dose ova feeding and the majority of these express Foxp3.[24] Despite these findings, the requirement of PPs in oral tolerance induction remains controversial. Studies using PP-null mice showed normal induction of oral tolerance, and only the removal of MLN before tolerization could prevent oral tolerance induction.[25,26] Moreover, although suppression of humoral immune responses to oral antigen was impaired and associated with a reduced number of T cells in the PPs in germ-free mice,[27] antigen-specific T-cell tolerance to oral antigens was not impaired in germ-free mice,[28] suggesting that there may not be a direct relationship between events in the PPs and antigen-specific tolerance to luminal substances. Together, these studies suggest that although PPs may be a site for antigen-specific tolerance induction to luminal substances, their role may be secondary to MLNs in this process. In contrast to the initial descriptions of the location of M cells, M cells also have been observed in the villous epithelium of the small intestine,[29] raising the possibility that M cells might be required for the transcytosis of luminal macromolecules across the non–follicle-bearing epithelium to initiate adaptive immunity. Moreover, M cells could deliver luminal macromolecules to DCs within the PPs or lymphoid follicles, which then migrate to distant sites to initiate adaptive immune responses. However, observations have suggested that PPs are not required to induce oral tolerance and that T-cell responses to oral antigens can be induced in the absence of M cells,[25,26,30] thus although M cells contribute to macromolecular transport and immune responses within follicles, their requirement for macromolecular transport contributing to immune responses in the MLN and oral tolerance is less clear.

In a landmark observation, it was identified that APCs within the lamina propria (LP) had the ability to extend dendrites between epithelial cells to capture bacteria without compromising the epithelial barrier.[31,32] Subsequent studies using ex vivo and in vivo imaging approaches on explants and living intestine from APC reporter mice showed LP-APC extension of transepithelial dendrites (TEDs) in the uninfected intestine and increased TED extension in response to infection,[33,34] raising the possibility that the LP-APC extension of TEDs plays a role in sampling the luminal substances in both the steady state and during infection (Figure 1). A combination of findings describing deficits in C-X3-C Motif Chemokine Receptor 1 (CX_3CR1) mice provided further support to the concept of direct capture of luminal substances by LP-APCs to support adaptive immune responses. CX_3CR1^+ LP-APC TED extension was impaired in the absence of CX_3CR1.[35] Moreover, studies found that tolerance to a dietary antigen was not effectively induced in the absence of CX_3CR1.[4,36] In combination, these observations implied that LP-APC directly capture luminal macromolecular substances in the steady state to support adaptive immune response. However, LP-APC TED extension was found to be absent in some mouse strains,[37] which do not show a deficit in the induction of T-cell responses to dietary antigens.[4] Furthermore, the absence of CX_3CR1, which impairs TED extension,[35] did not impair the stimulation of CD4+ T cells in the MLN in response to luminal antigen.[38] Together these observations indicate that although TED extension could directly deliver luminal substances to LP-APCs, the extension of TEDs is not required for the induction of antigen-specific T-cell responses in the steady state. Although the extension of TEDs may not be required for the induction of antigen-specific T-cell responses to luminal substances in the steady state, CX3CR1+ LP-APCs still may play a critical role in this process. CX_3CR1+ LP-APCs have been shown to capture luminal substances and transfer them to CD103+ LP-APCs to initiate T-cell responses to luminal substances and promote tolerance.[36] Thus, CX3CR1+ LP-APCs may contribute to steady-state responses to luminal substances in multiple ways.

Recently, it was identified that goblet cells can take up luminal high-molecular-weight substances and transfer them to LP-APCs in a process termed *goblet cell–associated antigen passages* (GAPs).[39] Interestingly, the endocytic properties of intestinal goblet cells to take up luminal substances has been noted for decades,[40–43] and intriguingly this property of goblet cells is being leveraged for oral drug delivery.[44–49] Observations support that LP-APCs acquiring luminal substances via GAPs are effective at inducing antigen-specific T-cell responses. When goblet cells and GAPs are absent or when GAPs are inhibited, LP-APCs cannot acquire luminal substances in a manner capable of stimulating antigen-specific T-cell responses in ex vivo assays.[39,50,51] Moreover, in the absence of GAPs, adoptively transferred T cells specific for luminal antigens do not expand or proliferate in the draining mesenteric lymph nodes in vivo.[51,52] Thus, goblet cells and GAPs have an essential role in delivering luminal antigens for the induction of T-cell responses outside of the organized intramucosal lymphoid tissues, the PPs, and isolated lymphoid follicles. Whether this property to take up and deliver luminal substances to support adaptive immune responses extends to other intestinal epithelial secretory lineages, Paneth cells, and enteroendocrine cells has not been fully explored. Similar to goblet cells, Paneth cells and enteroendocrine cell development is dependent on the transcription factor mouse atonal homologue 1,[53] and accordingly would be affected by strategies deleting mouse atonal homologue 1 in intestinal epithelial cells. Enteroendocrine cells have been observed to take up high

molecular substances from the gut lumen.[54] However, because of their slower turnover, Paneth cells and enteroendocrine cells still are present during the time frame of the experiments, showing that the loss of goblet cells abrogates luminal antigen acquisition by LP-APCs, suggesting that their contribution to luminal antigen delivery to LP-APCs for the subsequent generation of T-cell responses is limited.[55,56]

Regulation and Regional Differences in GAPs to Control Immune Responses to Luminal Substances

In the steady state, adaptive immune responses to the diet and commensal microbes are dominated by tolerance, which largely is mediated by Foxp3+ Tregs. Tolerance to these innocuous antigens is necessary to avoid inappropriate inflammatory responses because these substances are encountered in the setting of abundant inflammatory stimuli from microbial products. In addition, it has been proposed that harnessing oral tolerance can be an effective means of treating immunopathology in type 1 diabetes,[57] arthritis,[58] autoimmune encephalitis,[59] and other diseases.[60] In contrast, during enteric infection, the adaptive immune response shifts to an inflammatory phenotype to promote pathogen clearance and protective immunity. Indeed, inflammatory T-cell responses can be generated toward dietary and commensal gut microbial antigens encountered during enteric infections,[51,61] thus emphasizing the need to control the immune system's access to these innocuous antigens, which can be mediated by GAP formation. GAPs form in response to acetylcholine acting on the muscarinic acetylcholine receptor 4 on goblet cells.[39] Observations support that acetylcholine is largely not limiting and that GAP formation and subsequent luminal antigen delivery to LP-APCs is largely regulated via inhibition of goblet cell responsiveness to acetylcholine.[18,50–52] Whether the source of acetylcholine supporting GAP formation is neuronal, non-neuronal, or can come from both sources is unknown. The inhibition of goblet cell responsiveness to acetylcholine to form a GAP occurs via activation of epidermal growth factor receptor (EGFR) expressed in goblet cells.[51] Activation of EGFR in goblet cells suppresses the ability of goblet cells to respond to acetylcholine through muscarinic acetylcholine receptor 4 expressed by goblet cells to form GAPs.[50,52] Thus, GAP inhibition can be mediated by multiple stimuli activating the EGFR including by luminal EGFR ligands, microbial ligands signaling via Toll-like receptors (TLRs) and MyD88 to transactivate the EGFR, and IL1β binding the IL1R to activate MyD88 and transactivate the EGFR[50,51] (Figure 1).

When present, GAPs throughout the gastrointestinal tract have been observed to deliver luminal substances to LP-APCs. However, there are regional differences in some properties of GAPs. Although microbial sensing suppresses colonic GAP formation, goblet cell–intrinsic MyD88-dependent sensing of the microbiota does not suppress GAP formation in the small intestine (SI) likely owing to the lower level of TLR expression and higher expression of inhibitors of TLR signaling by SI goblet cells when compared with colonic goblet cells.[50] A small number of GAPs are present in the distal colon despite the presence of the abundant gut microbiota.[62] Whether this is owing to the dense mucus layer in the distal colon preventing sufficient amounts of microbial products from accessing the goblet cells to inhibit GAP formation or whether this is owing to GAP formation via other receptors and stimuli is not known. When present, GAPs in the proximal colon, but not in the distal colon or SI, translocate live commensal gut bacteria.[18,62] The basis for this differential ability to translocate bacteria is unknown but could be related to the relatively less abundant bacteria in the SI lumen and the less-penetrable mucus layer in the distal colon when compared with the proximal colon.[63]

The pathways controlling GAP formation are central to allowing the immune system to respond appropriately to luminal substances. When GAP inhibition by goblet cell–intrinsic microbial sensing is overridden in the proximal colon, inflammatory responses are generated, and live commensal bacteria translocate across the colonic epithelium.[18,62] Moreover, overriding GAP inhibition during enteric infection induces inflammatory T-cell responses to dietary antigens.[51] Perhaps the most striking example of the control of GAP formation occurs in the preweaning gut. During the first 10 days of life in nursing mice, GAPs are inhibited by EGF, presumably of maternal origin in breastmilk. After 10 days of life, when the luminal EGF concentration decreases, GAPs form first in the colon, then in the SI, and then are inhibited in the colon by the gut microbiota around the time of weaning. These orchestrated events allow for a defined interval between the 10th day of life and weaning, at which point tolerance to some commensal bacteria is established.[52] Moreover, altering the pattern of GAP formation preweaning has long-term effects, predisposing to colitis when commensal bacteria are encountered by the immune system later in life.[52] Although the role of GAPs in inducing tolerance to dietary antigens in the steady state has yet to be explored, these observations suggest that, when present, GAPs facilitate tolerance to dietary and commensal bacterial antigens and that GAP inhibition helps prevent inappropriate responses to dietary and commensal bacterial antigens encountered in hostile settings.

A Potential Link Between Gaps and Early Observations of Macromolecular Transport of Luminal Substances

Two phenomena of transport of macromolecular luminal substances were described decades ago, persorption,[14,64,65] which occurs in the adult intestine, and macromolecular transport in the neonatal intestine, which persists for a defined period, the cessation of which has been termed *closure*.[66–68] It is interesting to speculate how these earlier observations might relate to the more recent descriptions of goblet cell–mediated transport of macromolecular substances.

Studies have suggested that persorption occurs by engulfment of luminal macromolecules by columnar villous

epithelial cells, and based on morphologic appearance these epithelial cells may be undergoing apoptosis.[69,70] Whether these columnar epithelial cells included goblet cells or were restricted to goblet cells was not determined. However, although earlier studies suggested an association of apoptosis with persorption by morphologic appearance, GAP formation was not associated with goblet cell apoptosis as determined by immunofluorescence staining.[39] In addition, it has been observed that persorption was increased by antibody coating, suggesting that although this process may not require antibody coating, it may be facilitated by fragment crystallizable region (Fc)-receptor binding,[71] a process that has not been evaluated in antigen delivery by GAPs. In support of a link between persorption and GAP formation, persorption was inhibited by the panmuscarinic receptor agonist atropine and further induced by the acetylcholinesterase inhibitor prostigmine,[65] which increases acetylcholine levels. Thus, identical stimuli induce persorption and GAPs, potentially suggesting that the historical observations of persorption may have included macromolecular delivery by GAPs.

In the 1970s, studies documented the ability of neonatal gut of many mammalian species in uptake of globulins from maternal colostrum, which was believed to be essential for passive immunization. The phenomenon of macromolecular transport in the neonatal period is a property that largely has been attributed to the SI. This may be owing in part to the presence of vacuolated fetal enterocytes in the preweaning SI, which have the capacity to endocytose large quantities of macromolecules from milk.[66] However, whether the vacuolated fetal enterocytes in the SI transport the endocytosed substances across the epithelium for release to DCs underlying the epithelium has not been evaluated, and is at odds with observations that the LP-APCs in the SI cannot be loaded with luminal antigens in the neonatal period in mice.[52] Alternatively, if the macromolecular transport seen in the preweaning gut occurs in the colon, this could be consistent with the presence of colonic GAPs during a defined preweaning interval. Potentially in support of a link between the presence of colonic GAPs in a defined preweaning interval and increased intestinal permeability in the neonatal gut, in some mammals, closure occurs around the time of weaning, the time in which colonic GAPs become inhibited by the expanding gut microbiota, and closure is altered by artificial feeds,[67,68] which should alter colonic GAPs in the preweaning intestine.

Conclusions

To combat the danger of invasion by potential pathogens yet allow selective sampling of the luminal contents to promote immune homeostasis, mechanisms exist to control and maintain the epithelium as a selective barrier to the uptake of macromolecular antigens. Although multiple pathways exist by which substances can cross the epithelium, steady-state encounters by the immune system with luminal substances in a manner capable of inducing antigen-specific T-cell responses are dominated by GAP-mediated antigen delivery. It is intriguing to note that observations of the endocytic property of goblet cells and macromolecular transport in the gut foreshadowed identification of goblet cell–mediated antigen delivery by decades. Although much remains to be learned about this antigen delivery mechanism, observations support that it is highly regulated and central to gut immune homeostasis. During the past decade, significant progress has been made in our understanding of macromolecular transport across the intestinal epithelium. Although our knowledge of intestinal transport has increased, we lack an understanding of how these processes are altered by disease and by gut microbiota, and whether alterations in these processes contribute to disease pathogenesis.

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The authors disclose no conflicts.

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