



Intestinal Defenses—Maintenance of Intestinal Barrier Function in Host Defense

a report by

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The intestinal mucosa represents an important interface between host epithelial cells, microbes, and antigens. The epithelial cell lining forms a selective barrier that allows the transport of nutrients, while at the same time preventing uptake of noxious antigens and luminal bacteria. With an estimated surface area of more than 200m², the intestinal epithelium must adequately protect itself from potential pathogens. Protective mechanisms including physical, chemical, and immunological barriers are in place to maintain homeostasis. A breach in any one of these barriers by microbial pathogens can lead to disease. Enterohemorrhagic *Escherichia coli* (EHEC O157:H7) is a food-borne, pathogenic bacterium associated with abdominal cramping, diarrhea, bloody diarrhea (referred to as hemorrhagic colitis), and kidney failure due to the hemolytic-uremic syndrome. Recent outbreaks from consumption of contaminated spinach and water sources are examples of the importance of barrier protection against pathogenic bacteria.¹

Mucosal Barrier

Epithelial cells serve as the surface layer lining the gut and, as such, provide the first line of defense against threats from luminal antigens and microbes. It comprises a single layer of polarized, columnar epithelial cells supported by a fibroblast sheath. The underlying lamina propria contains a host of immune cells and nerve fibers, as well as endothelial cells comprising blood vessels. The rest of the gut wall is composed of the muscularis mucosa, the submucosal plexus, and the serosa.

Interspersed between epithelial cells along the crypt–villus axis are goblet, Paneth, and enteroendocrine cells, which are all vital in maintaining mucosal protection. Enterochromaffin (EC) cells are a subtype of enteroendocrine cells that produce serotonin in response to luminal stimulation. EC cells act as sensory transducers to luminal changes in acidity, osmolarity, and nutrients, including amino acids and free fatty acids,² as well as pathogens and bacterially derived toxins. Serotonin is contained in basolaterally located granules and released both constitutively and following stimulation into the lamina propria, where it can activate nerve fibers² and subsequently cause goblet-cell-derived mucin secretion and passive water flux to wash away noxious antigens. The congenital absence of EC cells in the intestine resulting from a mutation in the neurogenin-3 gene causes chronic malabsorptive diarrhea,³ highlighting the importance of these cells in maintaining fluid and ion transport.

Goblet cells are specialized epithelial cells that produce a viscous glycoprotein made up mainly of secreted and membrane-bound mucin glycoproteins.⁴ Mucus forms a protective layer above the surface epithelium to prevent adherence of luminal bacteria and provide lubrication for propulsion of gut contents. Along with mucins, the trefoil factor family (TFF) of peptides is secreted by goblet cells, and is also important in defense

and repair following injury.⁵ Specifically, TFF3 increases transepithelial resistance by increasing the expression of intercellular tight junction proteins in polarized epithelial monolayers.⁶

The apical surface of epithelial cells is covered with a rapidly turning over, complex glycocalyx network that projects from the apical microvilli on the surface of epithelial cells and can prevent the adhesion of luminal bacteria to the cell surface. Secretory immunoglobulin A (sIgA) antibodies produced by lamina propria plasma cells are also secreted with mucus and bind to noxious substances, such as bacteria and antigens, to assist in washing them away from the surface epithelium. Inflammatory cells and pathogenic bacteria can change mucin gene expression⁷ and effect maintenance of homeostasis. Micro-organisms penetrating the mucus layer are then exposed to cryptdins, a family of antimicrobial peptides released by Paneth cells that are important in innate host defense. Paneth cells are located near the base of the crypt, preventing colonization within the microenvironment of adjacent pluripotent stem cells.⁸ Together, these various cell types are integral in defending the host from luminal antigens and bacterial invasion.

Epithelial Fluid and Electrolyte Secretion

Following a breach of the physical barriers, pathogens have access to epithelial cells, which trigger additional protective mechanisms. An appropriate fluid environment in the intestine is essential for maintaining normal absorptive and secretory functions. Tight regulation of ion transport is crucial, as over- or under-activation of ion and water secretion is linked to a variety of disease states, including secretory diarrhea (e.g. from cholera toxin) and cystic fibrosis, respectively.⁹ Homeostasis is maintained at the level of the epithelium and occurs primarily due to osmosis via the paracellular route, through intercellular tight junction proteins and, secondarily, by specific epithelial cell molecular water

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channels—aquaporins. Altered aquaporin localization has recently been associated with *Citrobacter rodentium*-induced diarrhea in mice.¹⁰ The high capacity for ion secretion in the gut is a defense mechanism that serves to flush out pathogens and thereby prevent mucosal invasion.¹¹ Excessive ion secretion for a prolonged period of time, however, can result in life-threatening diarrhea (e.g. following *Vibrio cholerae* infection) in the absence of adequate hydration.¹¹ Cholera toxin causes an increase in cyclic adenosine monophosphate (AMP)-mediated opening of apical chloride channels (cystic fibrosis transmembrane conductance regulator, CFTR), driving water losses and thereby causing diarrhea.¹² Active ion transport regulates passive water movement, with villus epithelial cells being absorptive (via sodium) and crypt epithelial cells being secretory (via chloride). Ion transport across the epithelium is driven by electrochemical gradients, which are maintained by various mechanisms, including electrogenic sodium absorption, electroneutral NaCl absorption, and chloride secretion. Secretion of bicarbonate and potassium also occurs in the intestine; however, the main determinant of a lumenally directed osmotic gradient is the mucosal transport of chloride into the gut lumen. Accompanied by increased propulsive mechanisms, fluid transport is an important mediator of bacterial clearance.

Mucosal Barrier Function

The epithelial barrier restricts both paracellular and transcellular permeation from the lumen into the lamina propria. Permeability is regulated by numerous factors, including cytokines, hormones, and neurotransmitters. Inappropriate barrier function is associated with multiple intestinal and extra-intestinal diseases, including, for example, celiac disease, inflammatory bowel diseases, and atopic eczema. Despite the risk of injury to the host, transiently increased permeability may have beneficial effects, including increased antigen sampling leading to immune tolerance.¹³

Paracellular permeability in the gut is maintained by intercellular apical junction complexes, which are composed of tight junctions, adherens junctions, and desmosomes. Individual epithelial cells are linked by tight junctions at their apical poles, which permit ions to cross this barrier via pores created between their protein structures while maintaining cell polarity. Tight junctions are dynamic structures composed of transmembrane proteins, junction-adhering molecules (JAMs), and scaffolding proteins. Tight junctions confer the ability of the gut to maintain an electrically resistant monolayer, preventing the back-diffusion of ions and solutes.¹⁴ Occludins and claudins are two families of tetraspan proteins involved in the tight junction strands, although their precise role in maintaining their structure is only starting to be understood.¹⁴ When appropriately maintained, tight junctions regulate bidirectional transport of ions and proteins between adjacent epithelial cells, while at the same time excluding potentially noxious substances of greater physical size. Pathogenic bacteria can manipulate these junctional complexes for infection purposes.

Clostridium difficile toxin, for example, causes increased paracellular permeability via the disruption of tight junctions.¹⁵

Transcellular permeability of the epithelium is limited by transcytosis, the process involving endosomal uptake of proteins and subsequent transport across the entire cell. Epithelial cells contain lysosomes, which are important in the degradation of endosomal contents into peptides, and limit the transport of intact proteins across into the lamina propria. In contrast to paracellular permeability, transcellular permeability is directly associated with mucosal inflammation, accompanying bacterial (and toxin) uptake and translocation through the cell.¹⁶ Transcellular permeability is altered by acute,¹⁷ chronic,¹⁸ and early life stressors.^{19,20}

Innate Immune System

Bacterial antigens successful in breaching the physical barriers provided by the epithelium then trigger activation of the innate immune system, the early phase of immune activation, followed by subsequent activation of the adaptive immune system. Development of the innate immune response occurs in neonates and is highly dependent on maternal factors, including breast-milk feedings and birthing methods.²¹ The innate immune system is appropriately maintained by the normal intestinal flora (referred to as colonization resistance) via Toll-like receptors and Nod (nucleotide/binding oligomerization domain)-like receptors. Toll-like and Nod-like receptors are the main communication mechanism between bacteria and the host, allowing physiological inflammation to be maintained.²² These pattern recognition receptors recognize specific bacterial components and trigger activation of the innate immune system accordingly.²³ Beneficial, probiotic bacteria within the colonic flora can also help dampen host immune responses and thereby maintain homeostasis. Certain pathogens, including EHEC O157:H7 infection, negatively modulate host signaling cascades, including, for example, interferon gamma-mediated signal transduction,²⁴ in an attempt to evade host immune responses.

Conclusions

The intestine is a complex organ that maintains a delicate balance between nutrient absorption and protection against noxious antigens. Key factors to maintain this barrier are in place to ensure that this function is appropriately carried out, with alterations leading to disease states. Following exposure to pathogenic bacteria such as EHEC O157:H7, both secretory and inflammatory responses are initiated as the host attempts to clear the pathogen and prevent the development of systemic disease. ■

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