

Gastrointestinal tract barrier function

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The pig interfaces with its external environment at multiple sites including the mucosae of the airways, oral cavity, genitourinary tract, the skin and the gastrointestinal tract (GIT). Mucosae are also the primary sites at which the mucosa-associated lymphoid tissue (MALT) is exposed to and interacts with the external environment. The extent and magnitude of these interactions is greatest in the GIT, which has the largest mucosal surface and is also in continuous contact with the microbiota and dietary antigens. Consequently, the mucosal surface in the GIT is a crucial site of regulation for innate and adaptive immune functions.

In the GIT, the epithelium (consisting of epithelial cells, or *enterocytes*) lining the mucosa is the central mediator of interactions between the MALT and the external environment primarily because epithelial cells establish and maintain a *barrier*. The barrier in the GIT is more permeable (leaky) than in the skin, for example, but supports myriad of physiological functions including fluid exchange and ion transport. In addition, mucosal permeability is adaptable and may be regulated in response to extracellular stimuli such as nutrients, cytokines and bacteria.

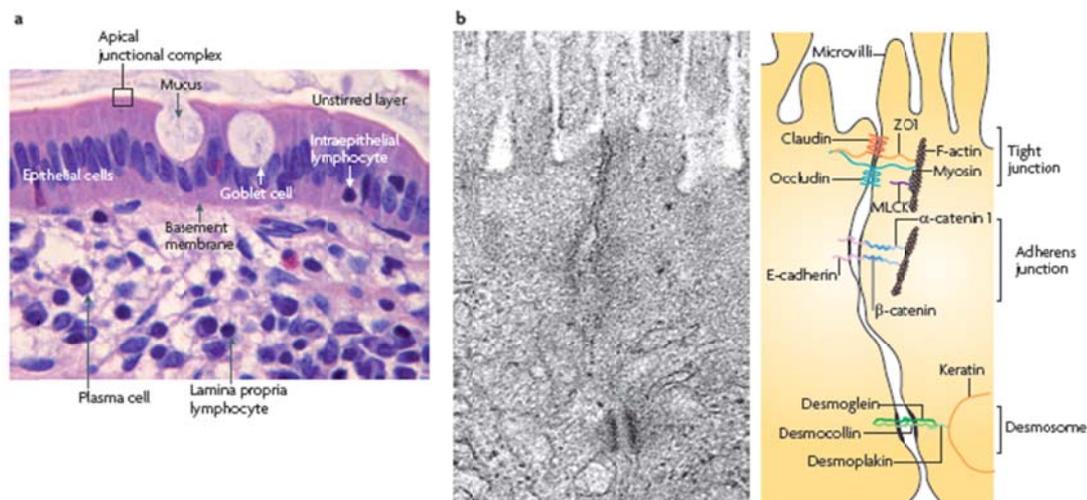
Anatomy of mucosal barriers

The mucosal surface of the GIT is covered by a hydrated gel formed by mucins secreted by specialized cells (such as Goblet cells) to create a barrier that prevents large particles, including most bacteria, from contacting the epithelium directly. Although small molecules can pass through the mucus layer, the bulk flow of fluid is limited and thereby contributes to the development of an *unstirred layer* of fluid at the cell surface. This reduces the rate of diffusion of ions and small solutes across the epithelium. In the stomach, this property works with HCO_3^- secretion to maintain alkalinity at the mucosal surface and prevent acid erosion of the cells. In the small intestine, the unstirred layer decreases nutrient absorption by reducing the rate at which nutrients reach the brush-border membrane of the microvillus, but may also contribute to absorption by limiting the extent to which small nutrients released by the activities of brush border digestive enzymes are lost by diffusion into the lumen of the GIT.

In addition to this physical protection, the mucus layer also provides pathogen colonisation resistance by allowing adhesion of commensal bacteria, thereby excluding intestinal pathogens. For example, it has been shown that feeding probiotic strains such as *Bifidobacterium lactis* and *Lactobacillus rhamnosus* inhibited mucosal adhesion of *E. coli*, *Salmonella enterica* serovar Typhimurium and *Clostridium difficile* in the pigs' small and large intestine (Collado *et al.*,

2007). Furthermore, as neutral mucins mature they become more acidic and viscous and are highly resistant to the bacterial proteases, further underlying the importance of mucous layer thickness and mucin production for optimum intestinal barrier function.

The principal responsibility for GIT barrier function, however, relates to the impermeable nature of the plasma membrane of the enterocyte. Direct epithelial cell damage, for example that induced by mucosal irritants, causes a marked loss of barrier function. However, in the presence of an intact epithelial cell layer, the *paracellular* pathway between the enterocytes must be kept intact. This function is mediated by the apical junctional complex, which is composed of the *tight junction* and subjacent *adherens junction*. Adherens junctions are required for assembly of the tight junction, which effectively seals the paracellular space (Turner, 2009; Fig. 1).



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Figure 1. Anatomy of the mucosal barrier.

- a. The human intestinal mucosa is composed of a simple layer of columnar epithelial cells, as well as the underlying lamina propria and muscular mucosa. Goblet cells, which synthesize and release mucin, as well as other differentiated epithelial cell types, are present. The unstirred layer, which cannot be seen histologically, is located immediately above the epithelial cells. The tight junction, a component of the apical junctional complex, seals the paracellular space between epithelial cells. Intraepithelial lymphocytes are located above the basement membrane, but are subjacent to the tight junction. The lamina propria is located beneath the basement membrane and contains immune cells, including macrophages, dendritic cells, plasma cells, lamina propria lymphocytes and, in some cases, neutrophils.
- b. An electron micrograph and corresponding line drawing of the junctional complex of an intestinal epithelial cell. Just below the base of the microvilli, the plasma membranes of adjacent cells seem to fuse at the tight junction, where claudins and other proteins interact. Other proteins such as α -catenin 1 and β -catenin interact to form the adherens junction (after Turner, 2009).

Tight junctions are multi-protein complexes composed of transmembrane proteins, peripheral membrane (scaffolding) proteins and regulatory molecules (e.g.,

kinases) (Kim et al., 2012). The most important of the transmembrane proteins are the *claudins* but also include occludins and the cytosolic proteins zonula occludens (ZO-1, ZO-2 and ZO-3), which join the transmembrane proteins to the cytoskeletal actins. The tight junctions limit solute movement along the paracellular pathway, which is generally more permeable than the transcellular (or *transepithelial*) pathway and hence is the rate-limiting step and the principal determinant of mucosal permeability. Moreover, tight junctions show both size selectivity (e.g., there is limited flux of proteins and bacterial lipopolysaccharides whereas whole bacteria cannot pass) and charge selectivity for transport of substances, and these properties may be regulated individually or jointly by physiological or pathophysiological stimuli (Turner, 2009). For example, alterations of tight junction protein formation and distribution through dephosphorylation of occludins, redistribution of ZO, and alteration of actomyosin through phosphorylation of myosin light chains causes intestinal paracellular barrier dysfunction. Enteric pathogen and endotoxin translocations are known to increase paracellular permeability through tight junction alterations (Glenn et al., 2009).

Barrier function and their interrelationship to GIT mucosal homeostasis

The integrity of barrier function clearly is an important component of optimal GIT structure and function in the pig. This function is underpinned by relationships between luminal material such as that from the diet, external stressors, the microbiota and mucosal immune function. Key in this interrelationship are the roles of some cytokines, small signaling molecules released by activated immune cells, to regulate the function of the tight junction barrier. In the pig, this (homeostatic) relationship in which regulated increases in tight junction permeability or transient epithelial cell damage trigger the release of pro-inflammatory cytokines, such as TNF and IFN γ , occurs on a day-to-day basis and without any appreciable effects on production and health. Nevertheless this balance between heightened activation of inflammatory cascades (pro-inflammation) and immunoregulatory responses is precarious and can fail if there are exaggerated responses to pro-inflammatory cytokines, as can occur for example in the post-weaning period. Resultantly, mucosal immune cell activation may proceed unchecked and the release of some cytokines may enhance barrier function loss that, in turn, allows further leakage of luminal material and perpetuates the pro-inflammatory cycle. In this sense, loss of GIT barrier function is also associated with increased enteric disease risk, and this article will now discuss some events occurring in the post-weaning period to highlight their impacts on epithelial cell barrier function.

The post-weaning malaise and barrier function

Post-weaning stress, such as mixing and moving, and dietary effects, such as the loss of immunoprotective factors in milk and (or) post-weaning anorexia, play major roles in GIT health and defense. Early weaning stress in the pig, for

example, induces immediate and long-term deleterious effects on intestinal defense mechanisms including lasting disturbances in intestinal barrier function (increased intestinal permeability), increased electrogenic ion transport, and dysregulated intestinal immune activation (Moeser et al., 2007). In addition, diarrhoea caused by enterotoxigenic *E. coli* (ETEC) serotypes is a major cause of morbidity and mortality in pigs worldwide. Enterotoxigenic *E. coli* serotypes can colonise the small intestine of susceptible pigs and produce toxins including heat labile (LT), heat-stable (STa and STb), and enteroaggregative *E. coli* heat-stable enterotoxin-1 (EAST1). Toxins can penetrate the innate mucosal barrier to the microvillar space, and subsequent enterocyte activation and fluid secretion causes areas of barrier disruption. The subsequent increase in paracellular permeability decreases transepithelial electrical resistance of the small intestine, and the loosening of the tight junction due to ETEC infection can potentiate further the translocation of antigens and toxins into the circulatory system triggering inflammatory cascades (or immune system activation). The disturbed barrier, in turn, renders the intestine susceptible to pathogen attachment, leading to colonization, pathogen proliferation, and clinically apparent infection (McLamb *et al.*, 2013).

Nevertheless and in response to ETEC challenge, the young pig mounts an innate immune response to rapidly clear or contain offending pathogens to prevent prolonged inflammation and sepsis. This response is initiated by the recognition of bacterial ligands and activation of epithelial and resident sub-epithelial immune cells, such as mast cells, macrophages and dendritic cells, leading to a burst of pro-inflammatory cytokine production (e.g., IL-6, IL-8, and TNF- α) and lipid-derived mediators (e.g., prostaglandins, leukotrienes) into the surrounding tissue and circulation. Released pro-inflammatory mediators recruit effector cells such as neutrophils to the site of infection where, via multiple mechanisms, they aid in containing and eventually clearing the pathogen (McLamb *et al.*, 2013).

In this regard, work published by McLamb *et al.* (2013) demonstrated that pigs subjected to early weaning stress exhibited a more rapid onset and severe diarrhea and more profound reductions in growth rate, compared with late-weaned pigs (16 versus 20 days of age). Furthermore, exacerbated ETEC-mediated clinical disease in early weaning stress pigs coincided with more pronounced histopathological intestinal injury and disturbances in intestinal barrier function (increased permeability) and electrogenic ion transport. In accordance with the abovementioned information, these authors showed that ETEC challenge exacerbated clinical and pathophysiological consequences of early weaning stress in pigs coincidental with defects in intestinal epithelial barrier function and suppressed mucosal innate immune responses.

Summary

Significant progress has been made in understanding the processes by which physiological and pathophysiological stimuli, including cytokines, regulate tight junction function in the GIT. Recent data have emphasized the presence of immune mechanisms that maintain mucosal homeostasis despite barrier dysfunction, and some data suggest that the epithelium orchestrates these immunoregulatory events through direct interactions with innate immune cells. Future elucidation of the processes that integrate mucosal barrier function, or dysfunction, and immune regulation to prevent or perpetuate disease, such as that caused by enteric pathogens in pigs, may lead to novel therapeutic approaches for diseases associated with increased mucosal permeability. In association, management practices that reduce stress at critical periods during the pig production cycle will assist in alleviating the negative effects caused on barrier function.

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