

Epithelial Cell Biology/Barrier Function

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The epithelial barrier as the primary mechanism of host defense

The epithelium lining of the intestine is composed of a monolayer of cells such as transporting enterocytes, goblet cells, enteroendocrine cells, M cells and Paneth cells. These intestinal epithelial cells are joined together at their apical poles by tight junctions that are modulated in response to signals from the epithelium itself, the lamina propria or upon events in the intestinal lumen. The intestinal epithelial cells are actively engaged in digestion and absorption of nutrients, but also provide an effective barrier that excludes the antigenic and proinflammatory contents of the intestinal lumen and thus separates it from intraepithelial and lamina propria effector immune cells which are highly sensitive to these stimuli. The presence of abundant leukocytes in the intraepithelial and subepithelial compartments shows that even under normal conditions the intestinal mucosa is an active, carefully balanced immunological organ, which provides protective host mucosal defense without the destruction of intestinal tissue. The activation of the intestinal immune system is determined by the regulation of the intestinal epithelial cell barrier function and the interaction of intestinal epithelial cells with non-immune or immune cell populations mediated by cytokines and growth factors.

To function as an effective barrier against peptides and macromolecules with antigenic potential and microorganisms of the gut lumen, the intestinal epithelium is highly organized. Intestinal epithelial cells are polarized and stabilized by a cytoskeleton and can be separated in apical and basolateral domains that differ in molecular composition and function. Neighbouring cells are joined together by continuous circumferential tight junctions located at their apical contact surfaces. These intercellular junctions seal the paracellular space and stabilize the epithelial monolayer.

This structural primary barrier is supported by a pre-epithelial barrier consisting of mucus, trefoil peptides and glycocalyx. In addition, Paneth cells located at the base of the crypts in the small intestine release cell lysozyme, secretory phospholipase A₂, and -defensins to provide a biochemical barrier against microbial colonization. Furthermore, intestinal epithelial cells are also able to actively regulate mucosal immune response through the secretion of growth factors and immune modulators. In these intestinal immune responses commensal bacteria co-operate with the host immune system to inhibit the overgrowth with harmful bacteria and support the absorption of nutrients.

1. Regulators of epithelial paracellular channels, ion and water transport

The tight junction is a specialized membrane domain at the most apical region of polarized epithelial cells that not only creates a primary barrier to prevent paracellular transport of solutes (barrier function) but also restricts the lateral diffusion of membrane lipids and proteins to maintain cellular polarity (fence function). These tight junction complexes are macromolecular assemblies of proteins that form contiguous rings at the apices of epithelial cells. Most recently the molecular composition of tight junction has been elucidated in some detail and for the first time the tools (antibodies and gene knockout animals) are available to study this structure and its regulation in more detail in healthy and diseased intestine.

One emerging family of tight junction molecules are the claudins, which are tissue type and intestine segment specific expressed. They have the potential to regulate physical barrier function, but are also involved in ion and water transport. Most of the claudin family member genes have been identified in expressed sequence tag (EST) databases based on their nucleotide and amino acid homology. Consequently, the physiological function of claudin integration into tight junctions has not been determined for most of these proteins. Increasing evidence suggests that claudin expression is intestinal segment specific regulated with different claudins demonstrating distinct mRNA expression in gastrointestinal organ systems.

The human genome project has identified a large number of genes, whose products are potentially involved in the regulation of the cytoskeleton and junctional structures of intestinal epithelial cells, among those novel (membrane-associated guanylate kinase-like) MAGUK family members, and a large number small GTPases of the Rab family.

2. Regulation of epithelial membrane compartmentalization and polarization.

The membrane dynamics responsible for vesicular transport and protein sorting are fundamental to the structure and function of polarized intestinal epithelial cells and to their ability to maintain a selective barrier required for efficient solute transport and host

defense. Many microbial agents co-opt the mechanics of membrane internalization to enter the host, or to breach the epithelial barrier. The severe diarrhea caused by *V. cholerae* infection represents a prime example. Cholera toxin must enter the host cell by trafficking retrograde from the cell surface into the ER where the toxin is unfolded by chaperones endogenous to the host and dislocated out of the ER through the protein-conducting channel Sec61p, the translocon. In other well described instances, Immunoglobulins IgG and sIgA bind to specific trafficking receptors to move across the epithelial barrier as intact and fully folded proteins. In doing so, IgG and sIgA act in the intestinal lumen to participate in host defense and immune surveillance at this mucosal surface. Almost all aspects of epithelial cell physiology and biology depend on the proper sorting of membrane proteins and the establishment and maintenance of intracellular membrane compartments exhibiting specific structure and function. These fundamental aspects of cell biology dictate the phenotype of the mucosal barrier in health and disease. Such mechanisms of membrane trafficking also account for antigen processing essential to the host immune response. Both membrane lipids and membrane proteins follow specific and regulated trafficking pathways. Given the strict dependence of cell function on these aspects of membrane dynamics, considerable emphasis should be given to well conceived studies on the molecular mechanisms of membrane biogenesis, trafficking, and protein and lipid sorting.

3. Dynamics of cellular junctions in epithelial migration in normal intestine and in wound healing.

Epithelial discontinuities or “wounds” are a common occurrence in disease states such as inflammatory bowel disease. Such epithelial disruptions are deleterious since they expose underlying tissues to noxious substances in the lumen of the gastrointestinal tract. As a protective mechanism small epithelial discontinuities swiftly reseal by migration of epithelial cells also referred to as “restitution”. Migrating epithelial cells undergo a marked change in shape from their normal columnar phenotype as they flatten to migrate and cover denuded surfaces. However, unlike other cell types such as leukocytes, epithelial cells maintain intercellular associations with their neighbors, thereby migrating as a co-ordinate sheet of cells rather than individual cells. Intercellular associations therefore have to be modified so as to facilitate forward movement and wound closure. Migration of an epithelium requires a network of signaling events and coordination between the cytoskeleton, intercellular junctions and cell matrix adhesion receptors. Such biological events are in turn influenced by cytokines growth peptides and trefoil factors that are released by epithelial cells or stromal and immune competent cells in their vicinity during active inflammation. Study of these biological events is therefore vital for not only understanding disease processes but also for the development of therapeutic agents to facilitate re-establishment of the epithelial barrier.

4. Anti-microbial peptides, trefoils and mucous layer in IEC barrier defense.

Evidence supports the hypothesis that the release of gene-encoded antimicrobial peptides by epithelial cells contributes to innate mucosal immunity. For example, mouse MMP-7 (matrilysin) is the prodefensin processing metalloproteinase in mouse Paneth cells, and MMP-7 null mice lack mature alpha-defensins and are defective clearing of orally administered *E. coli* and increased susceptibility to virulent *S. typhimurium*. In

gastric epithelium and colon, certain beta-defensins are expressed constitutively and others are induced upon bacterial exposure by mechanisms that are not understood. The human cathelicidin LL-37 is expressed in colon and upregulated during active UC. The regulation of these biosynthetic and processing events have not been characterized.

Trefoil peptides, which comprise a family of small protease-resistant proteins characterized by one or more trefoil motifs, play a critical role in epithelial restitution. Three trefoil peptides have been identified in humans; spasmodic polypeptide (SP/TFF2), pS2/TFF1, and intestinal trefoil factor (ITF/TFF3). Human SP is mainly expressed in the gastric mucous neck cells, and pS2 is expressed in gastric surface (foveolar) mucous cells. ITF is synthesized and secreted by goblet cells in the small and large intestine. At the margins of mucosal injury such as IBD and gastric ulcer, the expression of trefoil peptides is rapidly up-regulated, and they appear to promote epithelial restitution. In ITF-deficient mice, epithelial restitution is absent in the colon, and oral administration of dextran sulfate sodium induces death associated with extensive colitis, presumably because of the failure of healing processes. Trefoil peptides have been shown to prevent gastrointestinal injury caused by alcohol and indomethacin and to induce rapid resealed of erosions. Thus, trefoil peptides play an important role in the repair and healing of gastrointestinal epithelium through enhancing epithelial restitution. Despite their potent protective role in epithelial integrity, the regulation of ITF expression and recognition remains to be elucidated.

The luminal surface of the gastrointestinal tracts are protected by the production of a thin mucus barrier. In its normal state the mucus is composed of 95% water, 2% glycoproteins, and 1% each of protein, lipids, and inorganic salts. The major structural components of mucus, which confer its viscoelastic properties, are glycoproteins called mucins. Mucins are classified as one of two main forms: membrane-associated or secreted. In general, a mucin subunit consists of a peptide core, making up 20% of the molecule, with diverse carbohydrate side chains comprising the remaining 80%. The side chains typically are highly sulfated and terminate in sialic acid. The size of a mucin subunit can range from 200 to 1,000 kD. In the intestinal epithelia and in the lower airways the goblet cell is a major producer of secreted mucins. Goblet cells can release large quantities of mucins in milliseconds in defense against acute insult. In response to chronic insult, they can increase in numbers leading to hypersecretion of mucus. With recent advances in the field of mucin biology it is now possible to look at specific mucin production. Nine human mucin genes and five rodent genes have been cloned or partially cloned to date, and thus antibodies and molecular probes have made it possible to look at message levels and protein expression of the specific molecules. It needs to be determined how mucins are regulated and secreted from intracellular granules to maintain basal epithelial protection. Furthermore, the environmental and inflammatory stimuli regulating enhanced mucin release are largely unknown.

5. Role of commensals and pathogens in the regulation of IEC development.

Pathogens have evolved a wide variety of mechanisms to interfere with distinct signaling pathways utilized to maintain IEC barrier function. In most cases, it is not clear

if the observed cellular responses are direct effects of bacterial mediators or expression of the compensatory response of the epithelial cells. However, the disruption of tight junctions with subsequent infiltration of the intestinal mucosa with pathogens will initiate an immediate immune response, which is characterized by the expression of a plethora of immune regulatory peptides. Until recently, it was not clear how these immune modulators affect the intestinal epithelial cell monolayer in its barrier function. It is also clear that intestinal epithelial cells 'sense' the content of the intestine through their integration into the innate immune system. The contribution of secreted defensins and other antimicrobial peptides to host defense is an open question, because it is unclear whether the peptides have only local effects against acute infection or interact with luminal bacteria to influence the composition of the enteric microbial flora.

6. Epithelial models for microbial pathogenesis

The task of the mucosal immune system guarding the gastrointestinal tract is immense. This system must be able to recognize and eliminate pathogens that enter the gastrointestinal tract without harming other functions. The epithelial cells lining the gastrointestinal tract form a highly specialized barrier that separates two very distinct environments, thus maintaining the delicate balance between the gut lumen and the underlying tissue. As part of its barrier function, the intestinal epithelium is able to detect surface-attached enteric pathogens and orchestrate a pro-inflammatory response that is largely responsible for diarrhea and other symptoms associated with intestinal inflammation. Some bacteria also see this as an opportunity to reach previous inaccessible places. The current paradigm indicates that intestinal epithelial cells respond to luminal pathogens by releasing distinctive pro-inflammatory chemoattractants, which sequentially orchestrate PMN movement across the intestinal epithelium. Transmigrating PMN disrupt the epithelial barrier function, which can allow access of luminal toxins and organisms to lymphocytes and other sub-epithelial cells that are not used to bathing in the noxious contents of the intestinal lumen. The responses of these cells to exposure to the luminal milieu will result in further promotion of the inflammatory state. Thus, microbial pathogens have evolved the capacity to engage their host in very complex interactions commonly involving the exchange of biochemical signals. Although much of this bacterial cross-talk maintains the well-being of the intestinal mucosa, enteric pathogens have found diverse ways to exploit the mucosa mucosal immune response to their own benefit. Since it is possible that inappropriate activation of the mucosal immune system can lead to chronic inflammatory disease states, understanding bacterial-epithelial cell interactions will likely contribute to understanding not only bacterial pathogenesis but also chronic inflammatory diseases of the intestine.

Although considerable effort has been directed at understanding the interactions of intestinal microbes with host cells, much of this work has utilized cultured cells of non-epithelial, or non-polarizing epithelial origin. However, the polarity of epithelial cells *in vivo* is an important aspect of their response to microbial pathogens. For example, epithelial cells infected apically with *Salmonella* can polarize the secretion of neutrophil chemoattractants both basolaterally and apically. For example, epithelial cells infected apically with *Salmonella* or enteropathogenic *E. coli* secrete neutrophil chemottractants

basolaterally Similarly, the mechanisms of Salmonella entry into polarized epithelia differ radically from those observed in non-polarized cell models. For this reason, when cell culture models are utilized for the study of enteric pathogens, emphasis should be placed on the use of polarized or polarizing cells. Important areas of research include the mechanisms of pathogen-induced effects on epithelial integrity, the transport of bacterial products across the epithelium, changes in epithelial gene expression induced by infection, modulating effects of commensal bacteria on pathogen behavior/biology, and epithelial restitution after pathogen-induced injury.

7. Signaling of the innate to adaptive immune system

The intestinal epithelium comprises an essential barrier that forms the frontline interface between mucosal surface and constituents of the intestinal lumen. It is, therefore, positioned to play a key role in the detection of the pathogen-associated molecular patterns involving both the complex variety of normal commensal bacteria, as well as those of superimposed pathogenic bacteria. The latter have been designated pathogen-associated molecular patterns (PAMPs) but products of many of these and other constituents also derive from the normal resident microflora, and thus might in many instances be properly designated CAMPs (commensal-associated molecular patterns). Although, intestinal epithelial cells (IECs) are constantly exposed to the broad spectrum of the resident microflora of the gut, little is known about the nature of the interactions between these cells and the high concentrations of luminal microbial products IECs constitutively express several members of a novel family of transmembrane receptors designated "Toll-like receptors (TLRs)" *in vitro* and *in vivo* that may serve as the pattern recognition receptors of the mucosal innate immune system to luminal CAMPs. The TLR family is comprised of at least 10 homologues of the *Drosophila* Toll protein. These receptors seem to function as a major link between innate and adaptive cellular immune gene responses in various mammalian cell systems. Recent studies provide compelling evidence that TLR4 serves as the main mediator of innate immune responses to LPS, whereas TLR2 may serve as the dominant cognate receptor for peptidoglycan (PGN). It is also presumed that these and other TLR family members that have yet to be fully characterized recognize distinct derived PAMPs/CAMPs. Downstream, LPS-induced signaling through TLR rapidly leads to nuclear factor- κ B activation and cytokine expression. However, the functional roles of the other TLRs and the possible collaborative interactions between different TLRs and other nonbacterial ligands, as well as the details of the TLR-induced cellular signal transduction pathways have not yet been fully defined.

Emerging as critical mechanisms for the recognition of PAMPs and CAMPs are intracellular receptors such as Nod1, Nod2. These are members of a growing family of cytosolic factors related to the apoptosis regulator Apaf-1 and a class of plant disease resistance proteins. Nod1 and Nod2 confer responsiveness to lipopolysaccharides and interact with RICK, a mediator of NF- κ B activation. Nod1 and Nod2 and related Nods appear to regulate the host response to pathogens, a process that may be faulty in certain inflammatory diseases. Recent studies that suggest that Nods may be involved in the recognition of pathogen components in the cytosol of mammalian cells. Mutations in the NOD2/CARD15 gene, recently identified on chromosome 16, have been

associated with disease overall but are found in only 25% of patients. It needs to be determined if the NOD family members are involved in mucosa mediated innate immune responses.

Recently, Paneth cells in small intestinal crypts were found to release defensin-containing secretory granules when exposed to bacteria and bacterial antigens. The components of these granules are considered to participate in innate enteric immunity, particularly within the lumen of small intestinal crypts. The receptors and signaling pathway(s) regulating these Ca²⁺-mediated events are unknown, but defects in these pathways may be predisposing factors in Crohn's. T cell activation induced by CD3 ligation and helminth infection result in respective 3-4-fold and 10-fold increases in the number of Paneth cells. The actual mechanisms and mediators of this alteration of epithelial lineage programming are unknown.

8. Epithelial cell interactions with inflammatory cells

It has become clear that epithelial function is regulated by the interaction of transepithelial migration of neutrophils, macrophages and dendritic cells. Clinical evidence suggests that PMN transepithelial migration diminishes epithelial barrier function, and it has been speculated that such alterations in epithelial permeability could have deleterious effects on vectorial ion transport. Moreover, PMN-mediated changes in permeability may provide a route of entry for molecules and/or microorganisms to the sub-epithelial space, thereby providing a mechanism for initiation and maintenance of inflammation. The molecular events underlying rearrangements of tight junctions by transmigrating PMN remain insufficiently understood. Studies utilizing transmigration inhibitors and PMN from patients with chronic granulomatous disease (a disorder in which PMN lack the ability to generate reactive oxygen species) suggests that the mechanism by which PMN migrate across tight junctions is not through proteolysis or oxidant production, but likely involves mechanical impalement of the tight junction. Some evidence indicates that at least one of the transmembrane proteins of the tight junction complex, occludin, may serve as a critical regulator of PMN trafficking through the paracellular space, and mutational analysis identified areas within the one of the extracellular domains important in regulating PMN transmigration. Such findings indicate the likelihood that PMN-epithelial crosstalk pathways exist in vivo and are pathophysiologically relevant to diseases of mucosal inflammation.

Identification of additional molecules involved in these crucial mechanism such as the junctional adhesion molecule (JAM) will provided the potential for new and important insight into paracellular trafficking of PMN and pathogens through the epithelium.

9. Epithelial development and stem cells

In order to completely understand the complexity of the developmental programs of the intestine, it is necessary to determine how normal adult intestinal epithelial stem cells are controlled, what controls stem cell number and function in the normal tissue, and what are the consequences of perturbing such control. The haemopoetic stem cells have been the most intensively studied of all adult tissue progenitors, with those of the epithelial tissues perhaps the next most-heavily investigated. Work is required in the identification and characterisation of adult intestinal epithelial stem cells and the

development of stem-cell-specific markers. Such markers are currently lacking, but their availability will enable the isolation and purification of these crucial cells. One of the major challenges of the next decade is to identify the genes, and hence proteins, that control their control during proliferation, self-maintenance and differentiation. In addition to providing novel therapeutic targets, these factors may permit the expansion of pure stem cell populations in vitro, facilitating gene therapy and tissue engineering approaches.

Furthermore, the field requires genetic model systems, which allow assessment of the contribution of particular intestinal epithelial cell populations in gut function and defense. Targeting strategies for regulated gene expression and in intestinal epithelial cells need to be developed and standardized.

10. Intestinal gene expression profiling database

A growing number of investigators is generating gene expression surveillance data from various model systems, intestinal diseases, and treatments trials in human and mice. Cross-referencing and compiling these into databases to obtain experiment numbers high enough for statistical analysis, will greatly aid the identification of intestinal candidate genes, clarify unspecific inflammatory responses, and may also help identify the source of the mRNA expression in conjunction with laser capture microscopy. Efforts in the development of improved software tools for analysis and database convergence will become an important part of biomedical sciences.