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Recent insights into the integration of the intestinal epithelium within the mucosal environment in health and disease

RS Blumberg[1], L Li[2], A Nusrat[3], CA Parkos[3], DC Rubin[4] and JL Carrington[5]

INTRODUCTION

The Workshop on Local Influences on Health and Repair of Intestinal Epithelium, held on 25–26 March 2008, brought together the latest research on environmental cues that influence the epithelium. With inflammation, changes to the epithelium include barrier disruption, altered morphology, and metaplasia and cancer. The epithelium and its stem cells both influence and are influenced by the immune system and multiple other elements of the environment.

THE EPITHELIAL BARRIER

Intestinal epithelial cells (IECs) function as barriers between two biological compartments and regulate vectorial transport of ions and solutes between those compartments. These functions require the maintenance of epithelial structural integrity through cell–cell and cell–extracellular matrix adhesion, and polarized targeting of transport and channel proteins to functionally different apical and basolateral plasma membrane domains.[1] Polarized targeting of proteins is regulated by differential sorting and trafficking in the exocytic and endocytic pathways.[2] Little is known about how the cellular machineries involved in polarized sorting, trafficking, and delivering membrane proteins are regulated. Defining mechanisms regulating cell polarity is critical for understanding the epithelial barrier during normal physiology and in disease states of epithelial tissues.

Key epithelial intercellular junctions that are critical in the establishment of the epithelial barrier include the tight junction (TJ), adherens junction, and desmosomes. Despite the large inventory of intercellular junction proteins, how these proteins assemble and communicate to regulate epithelial barrier function is poorly understood. The TJ is a paracellular barrier that regulates movement of soluble material across the intestinal epithelium. Although there are more than 50 known TJ proteins, functions of most are undefined. Best understood is the claudin family of transmembrane proteins, which form continuous adhesive contacts and size- and charge-selective pores (~4 Å radius).[3,4] Several human diseases result from claudin mutations, most of which perturb epithelial barrier function. In chronic inflammatory bowel disease, a compromised epithelial barrier contributes to the perpetual mucosal inflammation. An improved understanding of intercellular junction proteins will also facilitate the development of therapeutic agents that can be delivered across the epithelium.

Intestinal mucosal barrier properties are also regulated by secreted products of Paneth cells that include α-defensins and additional host defense peptides.[5] Paneth-cell α-defensins have broad-spectrum antimicrobial activities and, in addition to defense against pathogens, are also predicted to influence composition of the enteric microbial flora.

THE DYNAMIC ROLE OF THE EPITHELIUM AND ITS LUMINAL AND CELLULAR ENVIRONMENTS IN PROMOTING MAINTENANCE AND REPAIR

The intestinal epithelium contains rapidly proliferating and continually differentiating IECs. Intestinal stem cells (ISCs) located in the crypts of Lieberkuhn give rise to dividing progenitor cells that undergo differentiation into the four principal cell types of the small bowel epithelium, including enterocytes and goblet, enteroendocrine and Paneth cells. This complex process of proliferation and differentiation is balanced by apoptosis in the crypts, and anoikis on the villus tips.

The gut epithelial luminal and cellular environments are remarkably heterogeneous. Interactions between the emerging epithelium and mesenchyme have been well studied in embryonic life, but

[1]Gastroenterology Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA. [2]Stowers Institute for Medical Research, Kansas City, Missouri, USA. [3]Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia, USA. [4]Department of Medicine, Washington University School of Medicine, St Louis, Missouri, USA. [5]National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, USA. Correspondence: JL Carrington (carringj@mail.nih.gov)

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have only more recently been the subject of intense scrutiny in the mature epithelium, and following epithelial injury. The development of culture methods for developing and mature intestine would yield important tools for studying the interactions between IECs and each of the environmental components for epithelial maintenance and repair.

Cross talk between the epithelium and lamina propria components, including myofibroblasts, fibroblasts, and smooth muscle cells, plays an important role in epithelial healing following a variety of injuries including inflammation, ischemia, resection, and radiation. These interactions are best exemplified in studies of the Hedgehog (Hh), bone morphogenetic protein (Bmp), and Wnt signaling pathways. Although it is clear that Hh, Wnt, Bmp, and Notch pathways play critical roles in regulating IEC proliferation and differentiation, expression and receptivity to these signals are exquisitely regulated in a temporal manner. Enteroendocrine cell differentiation exemplifies this regulation.[6] In addition, extracellular matrix and vascular and neural elements influence IECs. The luminal environment, including ingested nutrients, pancreaticobiliary and intestinal secretions, and the microbiome/commensal bacteria, also influences epithelial growth and differentiation.

Myofibroblasts play a key role in epithelial healing and repair. These cells express a panoply of cytokines, chemokines and growth factors, and their receptors. Their ability to secrete growth factors such as amphiregulins, bone morphogenetic proteins, a subset of Wnt ligands, fibroblast growth factor-2 and -7, insulin-like growth factor-1, hepatocyte growth factor, and VegF-A and -B suggests their role in regulating cell proliferation.[7] Secretion of matrix proteins such as collagens, laminins, tenascin, and matrix metalloproteinases indicates a role in healing and tissue remodeling. As presented, a role for myofibroblasts in immune regulation has been postulated.

Studies of Hh signaling have been particularly instructive and serve as a model of epithelial–mesenchymal interactions in the developing and adult gut. Recent studies have indicated a role for Hh signaling in villus smooth muscle homeostasis, and in regulating IEC proliferation and villus morphology.[8] Hh signals are paracrine and emerge from the epithelium.

Interactions between the extracellular matrix and epithelium are dynamic and are characterized by unique microenvironments along the crypt–villus and anterior–posterior axes of the intestine. Compositional changes at the cell–matrix interface regulate epithelial proliferation, migration, and differentiation. For example, differential expression and processing of laminins and the laminin-binding integrin receptors are found along the crypt–villus axis.[9] Further definition of these microenvironments is critical to provide insight into their contribution to intestinal function in both health and disease.

The microbiome exerts strong influences on the gut epithelium, in the normal and injured intestine and in inflammatory bowel disease. In normal intestine, commensal bacteria activate homeostatic responses by epithelial and immune cells to permit coexistence of gut microbes with epithelium. These mechanisms include downregulation of bacterial receptors and induction of intracellular and secreted molecules that inhibit immune responses, among which interleukin-10 and transforming growth factor-β appear particularly important.[10]

Neural modulation of gut epithelial proliferation and healing is mediated by substance P and neurotensin, for example, by activating the epidermal growth factor receptor.[11] In addition, these neuropeptides have antiapoptotic effects. As presented, their action appears context-dependent, as they have been shown to be proinflammatory during acute colitis, but have healing effects in chronic colitic states.

RECENT INSIGHTS INTO INTESTINAL EPITHELIAL STEM CELL BIOLOGY AND ITS ADAPTATION TO ENVIRONMENTAL INFLUENCES

The pattern of epithelial structure in the gut is determined by epithelial–mesenchymal interactions involving bone morphogenetic proteins, Sonic hedgehog, Notch, and Wnt, largely studied for their roles in radial patterning of the gut. New research also intends to elucidate the anterior–posterior patterning of the gut. For example, the homeobox gene *Barx1* is required for stomach morphogenesis and specification of stomach mucosa by antagonizing Wnt signaling.[12] The boundary between intestinal and stomach epithelium is sharply defined by expression of this gene. Multiple additional factors are likely involved in patterning the gut in all dimensions and may be key to understanding both development and metaplasia.

In the adult, a limited number of ISCs per crypt gives rise to all types of mature IECs. The major developmental regulatory pathways continue to regulate ISC self-renewal, progenitor cell proliferation, lineage commitment, functional maturation, and cellular apoptosis.[13] The ISC position within the crypts has been controversial. Crypt base columnar cells initially were proposed as ISCs[14] and a marker, GPR49/Lgr5, specifically labels crypt base columnar cells that contribute to all four types of IECs.[15] On the other hand, DNA label-retaining cells have been localized as putative ISCs directly above the Paneth cells.[16] A recent model was proposed that label-retaining cells represent "reserved," and crypt base columnar cells represent "primed" ISCs. The primed ISCs directly contribute to epithelial cell regeneration, and the reserved ISCs may replace primed ISCs lost under environmental stress.[13] This model is consistent with recent evidence in the hematopoietic system.[17]

ISCs reside in a specialized microenvironment, or niche, that limits their proliferation and inhibits their differentiation. In the mouse intestine, the niche is located at the crypt base. Its cellular elements include myofibroblasts, capillaries, and autonomic nerve dendrites. Certain types of intestinal injuries inhibit epithelial proliferation. One cellular response to maintain proliferation (and thus maintain the epithelial barrier) is a repositioning of stromal cells in the niche that expresses high levels of Cox-2 and prostaglandin E2.[18] An important future goal is to decipher the cellular and molecular interactions between the niche components and ISCs during health and disease.

Stem cells hold promise for repairing damaged intestinal tissues. Toward this goal, steps have been taken to isolate and characterize ISCs. Sorting of "side population" cells yielded a side population fraction that is far from pure,[19,20] but it appears that the number of CD45-negative side population cells is a reasonable surrogate for stem cell number.[21] Development of better isolation methods and functional assays of ISCs remains a pivotal challenge.

THE EPITHELIUM AS A BYSTANDER, PARTICIPANT, AND ORGANIZER OF INTESTINAL INFLAMMATION

It has been increasingly recognized that the intestinal epithelium is an important component of host immunity with significant implications for both inflammation and cancer. Specifically, it is well established that the epithelium not only functions as a physical barrier, as described above, but also is actively involved in innate and adaptive immunity and thus immunosurveillance and immunoregulation. These various immunologic properties are directly integrated into barrier function through, for example, the regulation of TJ proteins. This concept has driven the emergence of a more physiological definition of epithelial barrier function. Primary disruptions of TJ protein function occur through, for example, deletion of junctional adhesion molecule expression,[22] or disruption of claudins by proinflammatory cytokines such as interleukin-4, interleukin-13, tumor necrosis factor, and interferon-γ promotes intestinal inflammation.[23] Factors derived from lamina propria cells that affect the IECs are modulated by intestinal inflammation and, under homeostatic conditions, are important for the maintenance of the stem cell compartment, cell proliferation (Wnt signaling), and cell-fate determination (Notch signaling).[24] As such, during intestinal inflammation, the epithelium likely becomes an important bystander in the development and promotion of inflammation.

It has also become appreciated that the epithelium is an important participant in both driving and protecting from intestinal inflammation.[25] This is nicely exemplified by the role of the epithelium in regulating its relationships with recruited leukocytes and its responses to the hypoxia that develops during inflammation. The epithelium is able to adapt to the adverse conditions associated with intestinal inflammation through, for example, the expression of anti-inflammatory and/or barrier-promoting factors (e.g., interleukin-10, transforming growth factor-β, intestinal trefoil peptides, hypoxia-inducible factor, and adenosine metabolites). Many of these factors are a part of specific transcriptional programs in response to the local inflammatory milieu like hypoxia and may be amenable to manipulation, such as through inhibition of von Hippel-Lindau factor function, which negatively regulates hypoxia inducible factor, which normally protects the barrier from inflammation.[26]

An especially exciting notion is that the epithelium can actually organize mucosal immune responses and, thus, an intestinal inflammatory response. Several nascent observations have pointed toward this possibility.[27] Moreover, these effects of the IEC are observed in response to microbes that normally reside within the lumen and likely include the commensal microbiota itself showing how the IEC functions as a sentinel cell of the immune system, having the ability to interrogate the microbial composition of the intestinal lumen and elicit responses.[10]

What is not clear is whether primary alterations (i.e. genetically endowed) in IEC function can organize and/or protect from mucosal inflammation. Recent insights from the genetics of inflammatory bowel disease susceptibility have raised this possibility.[28] Targeted association studies have led to the identification of the *NOD2* gene for Crohn's disease, *MYO9B* for ulcerative colitis, and *MST1* gene for both Crohn's and colitis.[28–31] Genome-wide association studies have identified *ATG16L1* and *IRGM* genes as genetic risk factors for Crohn's, evidence that autophagy plays an important role in gut homeostasis.[32] Additional studies have implicated multiple other pathways and genes,[33] with the biological roles in inflammation and disease yet to be fully understood. One pathway that has raised recent interest is the unfolded protein response, which protects cells from endoplasmic reticulum stress associated with unfolded or misfolded proteins. Endoplasmic reticulum stress, similar to hypoxia, which may in and of itself promote endoplasmic reticulum stress, is associated with intestinal inflammation.[34] It can be envisioned that an inadequate endoplasmic reticulum stress response within the intestinal epithelium may increase susceptibility to inflammatory bowel disease.

RECENT DEVELOPMENTS IN INFLAMMATION AND CANCER IN THE INTESTINE

Development and progression of cancer is a serious consequence of chronic inflammatory processes of epithelial organs. Highlighted in this workshop, inflammation-associated neoplasia is highly influenced by the microenvironment and includes contributions from IECs, mesenchymal cells, and leukocytes. Epithelial contributions are diverse, ranging from aberrant Wnt pathway signaling to microbial stimulation of epithelial Toll-like receptors. Dysregulation of Wnt signaling is a major contributor as most colorectal tumors are initiated by APC gene inactivation and additional mutations in the genes encoding RAS, transforming growth factor-β type II receptor, p53, and others. It is now becoming apparent that activation of ISCs during inflammation may also play an important role in transformation that is preceded by architectural changes in the gut epithelium. Identification of key events for such activation such as phosphorylation of β-catenin through Akt may serve as biomarkers to monitor the status of ISC activation and the potential for subsequent neoplastic transformation.[35] Other epithelial effectors include luminal microbial product stimulation of Toll-like receptors resulting in activation of nuclear factor-κB and cyclooxygenase-2. Evidence was presented implicating aberrant expression of epithelial Toll-like receptor-4 as a potential contributor toward dysregulated proliferation.[36] It is also increasingly apparent that inflammation-associated mesenchymal cells are critical in the transformation and invasion processes. Specifically, mechanisms by which matrix metalloproteinases stimulate

epithelial–mesenchymal transition to increase fibrosis as well as generation of a tumor microenvironment were discussed, yet much needs to be learned. As an example, mesenchymal matrix metalloproteinase-3 production has been linked to increases in the small GTPase Rac1b to stimulate reactive oxygen species generation resulting in cell scattering and invasion.[37] Furthermore, data now exist demonstrating that novel subsets of immature myeloid cells are recruited from the bone marrow to the tumor-invasion front. These $CD34^+$ immature myeloid cells express specific matrix metalloproteinases and CC chemokine receptors such as CCR1, and migrate toward ligands expressed on the tumor epithelium. Manipulation of CCR1 expression decreases immature myeloid cell recruitment and suppresses tumor invasion.[38]

SUMMARY AND CONCLUSION

Research is moving rapidly to understand the factors that influence development, maintenance, and healing of intestinal epithelium. This research is crucial to understand diseases and conditions related to loss of epithelial integrity and barrier function. Because this epithelium is continually replaced, the factors that affect ISCs and direct proliferation and differentiation of their progeny are of intense interest. Methods for isolation, culture, and *in vivo* grafting of ISCs and their progeny would be invaluable tools. In addition, many factors that affect the adult system are also common to development. Thus, the similarities and differences between adult maintenance and embryonic/early postnatal development of the epithelium; the effect of timing and location of various influences within the microenvironment; how factors such as Notch and Wnt, which are pivotal to so many systems, are able to elicit system-specific responses; and the interactions between these various effectors are all prominent research questions for the future. The multiple roles of the cells and molecules in the lumen, the lamina propria, and from the immune system should not overshadow the role of the epithelium, itself, in regulating its own environment and modulating inflammation. Research to elucidate the complex two-way discussion between the epithelium and its environment promises exciting clues for development of therapies to calm inflammatory conditions and promote normal maintenance and healing of the epithelium.

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DISCLOSURE

The authors have no conflict of interest to disclose.

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