Introduction for the special issue on “Tissue Barriers in Inflammation”

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Abbreviations: IBD, inflammatory bowel disease; AJ, adherens junction; TJ, tight junction; PKC, protein kinase C; LPS, lipopolysaccharide; ICAM-1, intercellular adhesion molecule-1; PECAM-1, platelet endothelial cell molecule-1; NEC, necrotizing enterocolitis; HIF, Hypoxia-inducible Factor; GI, gastrointestinal; ER, endoplasmic reticulum

This issue of *Tissue Barriers* contains the inaugural special issue devoted to recent advances in barrier function of endothelial and epithelial cells. We used this opportunity to invite experts in vascular endothelial cell biology and epithelial cell biology to comment on critical questions and problems in permeability of organ and tissue barriers, and to provide insight into common areas in these fields, namely how these cells maintain homeostasis and response to injury and infection. To complement these reviews, this issue also contains four research articles that explore specific questions related respiratory and intestinal epithelial cell function.

The epithelial layer is the barrier for cutaneous, respiratory and gastrointestinal tissues and is the first line of defense against environmental (ultraviolet radiation, microorganisms and physical trauma) and endogenous (inflammation, cancers, ischemic/reperfusion injury and other injuries) factors. For example, the epithelium of the gastrointestinal tract provides an essential barrier function that selectively regulates fluid and solute exchange, interacts with both the adaptive and innate immune system, and endogenous microbial mass to maintain homeostasis. Compromised epithelial barrier has been linked to pathogenesis of a number of diseases including chronic inflammatory states and cancer. The endothelium is a selective and semi-permeable single cell monolayer that lines the entire vascular system and maintains a non-adhesive, non-thrombogenic surface to deliver nutrients and oxygen to tissues and organs. Damage or injury of endothelial barrier function is implicated in the pathogenesis of many diseases including atherosclerosis, diabetes, pulmonary edema, ischemic stroke, neurodegenerative disorders, sepsis and cancer. An important function of the endothelium is localized recruitment of circulating blood leukocytes to sites of injury, infection or immune mediated responses in a process called the “inflammatory response”. Once leukocytes extravasate the blood vessel wall into the interstitial space, chemotactic gradients composed of a variety of molecules entice leukocytes to the source of inciting agents, often involving subsequent transepithelial migration as in inflammatory bowel disease (IBD).

Epithelial and endothelial cells are specialized for the organs and tissue in which they reside. Both cell types exhibit significant plasticity in their phenotype and actively participate in the inflammatory response (**Fig. 1**). Alterations in barrier function in the endothelium and epithelium are often linked to the release of soluble mediators from resident cells (e.g., mast cells, macrophages, T cells, dendritic cells) and from recruited leukocytes and platelets. The importance and critical roles of the endothelium and epithelium are underscored by the sheer number of citations for each cell type 145,657 and 324,466 respectively, in PubMed as of January 2015. Herein, this issue contains twelve review articles and four basic research articles devoted to the fields of vascular, cutaneous, respiratory or gastrointestinal biology and their role in barrier function and adhesive interactions with innate and adaptive immune cells.

The vascular endothelium integrates several interrelated pathways to achieve proper barrier function at rest and during response to injury or infection. The molecules and mechanisms that maintain vascular barrier function are reviewed in concise detail by Rodrigues and Granger.¹ They provide a very informative description of the known cell-to-cell lateral junction complexes, such as adherens junctions (AJ) and tight junctions (TJ), and the cytoskeleton employed by the vascular endothelium to establish baseline barrier and illustrate the contributions of blood cells and their secreted products to endothelial cell barrier function. They place particular emphasis

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Figure 1. Neutrophils are recruited from blood vessels into inflamed tissues (steps 1-6) through a multistep process. For review see reference.¹⁸ The multistep adhesion cascade requires blood neutrophils to initially attach and roll on activated endothelial cells (step 1), arrest (step 2) and undergo directed transendothelial migration between adjacent endothelial cells (step 3) and then migrate between pericytes that envelope the vessel wall. By following chemotactic gradients emigrated neutrophils migrate through the interstitial space (step 4) towards the site of pathogenic *Salmonella Typhi* bacteria in the lumen of the intestine. This latter process involves neutrophil adhesive interactions and migration through the basement membrane followed by migration on the basolateral epithelial surface and then through lateral borders of the epithelial monolayer (step 5), and finally, localize to the bacteria that have colonized in the small intestine (step 6).

and downstream signal pathways. They explore the question “how and why” vascular leakage occurs in acute inflammation. The authors describe important clinical observations and provide an overview of current concepts and data sets on angiopoietins and Tie-2 stimulation that both initiate and resolve vascular leak during acute inflammation. The overall body of evidence presented tips the scale in favor of Angpt-1 promoting endothelial barrier in the context of infection, while Angpt-2 is deleterious in sepsis. Sarelius and Glading⁴ pick-up the torch and critically evaluate the contributions of integrin-mediated and endothelial immunoglobulin superfamily cell adhesion molecules (ICAM-1)-mediated signaling that regulate AJs VE-cadherin adhesion stability, endothelial barrier function, and vascular permeability. They provide a detailed synopsis and elegant illustrative figures on the known and putative crosstalk signaling mechanisms between integrins, ICAM-1 and PECAM-1, and inter-endothelial cadherin adhesions, as potential strategic signaling nodes for multipartite regulation of cadherin adhesion. Finally, the review by Dr. Simkin examines the pathways of microvascular permeability through the lens of the synovial tissue microvessels and synovial fluid in normal and arthritic knees.⁵ Experimental approaches are described that enabled investigation of the permeability of synovial endothelial cells to plasma proteins through the analysis of a limited number of normal and arthritic patient serum and synovial fluids and comparison to known measurements of lymphatic efflux and synovial plasma flow. A three pore model is proposed, consisting of small fenestral apertures, larger paracellular clefts and large pore, non-selective gaps. Interestingly, in arthritic (and presumably inflamed) tissues, Simkin reports that fenestral apertures widen and large pores open to permit significant increase in permeability, resulting in loss of barrier function. Prior reports on pore structure in microvessels of other tissues advance a two pore system.⁶ However, further work is needed to arrive at a more complete picture of the anatomic and molecular mechanisms that regulate barrier function in the microcirculation. Moreover, additional studies are necessary to understand at the transcriptional level the mechanisms that turn off the inflammatory response in endothelium and enable return to the resting baseline barrier function. Recent work on the lipid molecules produced by leukocytes including resolvins, lipoxins, protectins and maresins families, collectively called specialized pro-resolving mediators by the Serhan lab has shed considerable light on this exciting topic.⁷

on the effects of neutrophils, lymphocytes, monocytes, and platelets, and their secreted products on disruption of barrier function, and provide insights into new targets for therapeutic intervention.

The next four reviews bore into factors that contribute to maintenance as well as disruption of barrier (increase in permeability, also termed hyperpermeability). The article by Kasa and colleagues² provides an in depth review of the actinomyosin and microtubule cytoskeletal systems, the cytosolic adaptor proteins, and signaling modules located in AJs and TJs to vascular permeability function. This review focuses on the microvessels of the lung. Kasa and colleagues point out that microvessels, rather than macrovessels are key targets of edemagenic agonist that disrupt barrier function. The authors provide an insightful overview of recent literature on signaling pathways downstream of thrombin and Gram-negative lipopolysaccharide (LPS) that increase vascular permeability primarily. This occurs through cytoskeletal rearrangement leading to an endothelial contractile response, and ultimately, to disruption of endothelial cell-to-cell contacts, endothelial cell - matrix contacts, and transcellular transport. In particular, how molecules such as CPI-17 (PKC potentiated inhibitory protein of 17 kDa) and caldesmon affect myosin light chain kinase activity and stathmin affects microtubules are of interest. In the second article, Milam and Parikh³ begin with a historical perspective on the intimate relationship between vascular permeability and acute inflammation in human patients, which dovetails and complements the article on the relationship between the cytoskeleton and junctional complexes and hyperpermeability written by Kasa and colleagues.² Milam et al. articulate with precision how angiopoietin (Angpt)-1 and Angpt-2 interact with the same receptor, Tie-2, expressed on vascular endothelium, and paradoxically, evoke opposing effects on endothelial cell barrier function. This discussion paves the way to their forward thinking assessment of endothelial permeability, namely hyperpermeability, through the Tie2 - Angiopoietin1 and -2 axis

The regulation of vascular permeability is conceptually and physiologically linked to epithelial barrier function, especially in the adsorptive portion of the intestinal tract and in the kidney reviewed in reference.⁶ Both the epithelium and endothelium play an active and essential role in the recruitment of innate and immune cells to sites of injury, infection and immune-mediated responses. The article by Halpern and Denning⁸ begins with an overview of the development of the gut and its defense mechanisms, which provides the appropriate background for the subsequent five review articles on the epithelium in health and in diseases of the skin and gastrointestinal tract (GI). The authors focus on necrotizing enterocolitis (NEC), a GI emergency in premature infants. NEC in newborns is a devastating illness and the causes are not well understood. Here the authors present an overview suggesting that an immature gut barrier contributes to the pathogenesis of NEC. Lastly, the authors summarize therapeutic strategies that may be brought to bear to promote optimal development of neonatal intestinal barrier function in order to reduce the incidence and severity of NEC. The review by Brandner and colleagues⁹ provides a comprehensive overview on tight junctions in healthy skin and keratinocyte culture systems. They discuss in detail the dynamic crosstalk that occurs between tight junctions and the cutaneous immune system. Finally the authors highlight several observations that implicate aberrant tight junction function contributes to the pathogenesis of a number of common cutaneous inflammatory and neoplastic conditions. The next review by Lüttig and colleagues explores the structure of claudin-2 and its role in compromise of TJ function and suggest therapeutic targets to mitigate such effects.¹⁰ The TJs in distal regions of the GI tract contain claudin-1, -3, -4, -5, -7, and -8 and promote barrier function. In contrast claudin-2 is a TJ pore/channel forming protein and its expression is commonly increased in leaky epithelia such as the proximal intestinal epithelium. Thus, therapies to suppress pro-inflammatory cytokines and decrease claudin-2 expression could have beneficial effects in intestinal inflammatory disorders.

An important, and sometimes underappreciated, function of intestinal epithelial mucosa is secretion of mucus by goblet cells. Mucus forms a protective barrier that prevents microorganisms and injurious substances from reaching the surface of the epithelium. The review by Cornick, Tawiah and Chadee, provides a comprehensive overview of mucins including studies in mice lacking specific mucin genes (Muc2) and also includes insightful summary figures.¹¹ Mucus is a gel-like substance composed of 21 different mucins (mucopolysaccharides) that are produced and stored in cytosolic granules of goblets cells. The authors discuss the role mucins play in binding to and retaining commensal and potentially pathogenic (e.g., *Vibrio cholera*, EPEC) bacteria, and in providing immune-related functions through indirect and direct interactions with immune cells within the gut. The relationship between alterations in the mucus barrier, biosynthesis, and ER stress and IBD is thoughtfully discussed. In the next article Sumagin and Parkos provide a comprehensive review of the mechanisms underlying neutrophil migration across mucosal barrier in the context of inflammatory bowel diseases.¹² The authors focus on the role of epithelial expressed adhesion molecules in this process. Notably, neutrophils exit the blood vessel in an apical to basolateral direction, whereas neutrophils travel in the opposite direction, basolateral to apical route, to cross the epithelial barrier. Neutrophils are envisioned to migrate across the epithelium at cell-to-cell lateral borders, similar to vascular endothelium, and hence neutrophils have intimate contact with multiple lateral membrane complexes. A detailed story ensues describing the junctional complexes that neutrophils “negotiate” with to gain access to the intestinal lumen, as well as the surface molecules of the apical surface that mediate adhesion. These molecular interactions enable bidirectional communication between the neutrophil and epithelium, and the nature of these conversations and the pathophysiological implications are discussed at great length.

Low oxygen tension and fluctuations occur in the GI tract, particularly in the distal bowel. Under conditions of low oxygen, the transcription factor Hypoxia-inducible Factor (HIF), becomes stabilized and initiates transcription of multiple genes that promote cell survival. The review by Colgan and colleagues focuses on HIF in intestinal epithelium and its relationship to the adenine and creatine kinase metabolism pathways in the healthy mucosa and in patients with inflammatory bowel disease.¹³ The article illuminates the importance of adenine nucleoside metabolism, cell surface ecto-enzymes like CD73 and CD39 and the surface receptors that recognize their enzymatic products to regulate their expression by HIF1 α during hypoxic episodes. These molecules also function in the endothelium within the mucosa and submucosa of the intestinal tract, highlighting the concept that “healthy” endothelium is intimately linked to a healthy epithelial barrier. The main sources of ATP turns out to be neutrophils, platelets and endothelial cells, thus making connections to innate immune cells (see the above Sumagin reference).¹² It is noteworthy that epithelial barrier protective pathways driven by HIF1 α tend not to be the classical regulators of barrier function, such as the tight junction proteins. Rather, HIF-regulated pathways control overall tissue integrity.

Research Articles

Cytokines, their receptors and downstream cytosolic signaling systems are key pathways that enable cells to communicate with one another. The NF- κ B transcription factor is one of the key mediators of inflammation in many cell types. On the other hand NF κ B is also an important factor that controls homeostasis of many cells in the immune system. The idea that NF κ B plays a role in respiratory epithelial barrier function has not been examined to date. The research article by Ward and colleagues hypothesized that NF κ B inhibitors would increase alveolar epithelial permeability through dysregulation of tight junction proteins.¹⁴ Interestingly, their results suggest constitutive activity of NF κ B in cultured rat alveolar epithelial type I cells is necessary to maintain basal barrier functions, although the signaling pathways remain to be identified. In contrast, challenge of monolayers with LPS endotoxin led to loss of barrier (increased permeability) that was prevented by inhibitors of NF κ B. Here one can appreciate that context is a critical factor that

dictates the roles of NFκB. The second research article delves into the biological impact of cytokines secreted by T cells in a murine colitis model. The recruitment of specific T cell subsets is known to be a key event in immune-mediated diseases. T helper type-9 cells (Th9 cells) develop from naïve T cells under the influence of IL-4 and TGF-β and are characterized by the production of IL-9. IL-9 has pleiotropic effects on a number of different cell types. In the research article by Gerlach and colleagues, the authors tested the hypothesis that IL-9 is important in T cell mediated colitis in the hapten TNBS.¹⁵ Mice deficient in IL-9 showed much less influx of T cells in the lamina propria that was associated with changes in several TJ molecules. Intriguingly, the levels of claudin-2 were similar in both IL-9^{-/-} and WT mice, which is perhaps unexpected given prior reports (see reference¹⁰ in this article). The implication is that IL-9 secreting cell are involved in the pathogenesis of TNBS induced colitis, and raises the question of what effects IL-9 have on the inflammatory response in this model.

The mechanisms of microparticle transcytosis and delivery to antigen presenting cells (APC) were studied in intestinal Peyer's patch epithelium tissue sections derived from double transgenic mice with cytoplasmic expression of dsRed in M cells and EGFP in dendritic cells. These reporter fluorescent proteins enabled Sakhon and colleagues to label the cytoplasm of each cell type, and thus define boundaries of these cells and track the movement of basolateral vesicles associated with mucosal M cells (termed "M cell-derived microvesicles") to the subepithelial space.¹⁶ The authors employed fluorescent bacteria, synthetic particles, and soluble agonists to monitor movement from intestinal M cells to dendritic cells and also to compare this trafficking of M cell-derived microvesicles to transcytosed inert microparticles. These approaches and data set provide new insights into M cell – DC communication and suggest M cell-derived microvesicles are M cell structures potentially involved in immune surveillance of the intestine. In the final article of this issue, Zhang and colleagues investigated the role of bacterial secreted effector protein, AvrA in colonic epithelial cells in murine colon epithelium and in vitro cultured human Caco2 BBE epithelial cells.¹⁷ Of interest is their observation that *S. Typhimurium* carrying the *AvrA* gene enhanced, rather than reduced the protein levels of the TJ and their location in colonic epithelium. The mechanism was found to involve *AvrA* targeting of the c-JUN and JNK pathways, which in turn, stabilized TJ protein ZO-1 and suppressed AP-1 transcription factor activity.

In summary, the past decade of research has provided a significant advancement in understanding the molecular and cellular mechanisms that create and maintain barrier function in the endothelium and epithelium. These findings should enable future research to develop novel therapeutic treatments with better selectivity and function in treating inflammatory diseases. The guest editors are indebted to the authors for their time and expertise in providing outstanding articles that enhance our understanding of how endothelium and epithelium work together to maintain barrier functions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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