Epithelial adhesion molecules and the regulation of intestinal homeostasis during neutrophil transepithelial migration

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Abbreviations: PMN, polymorphonuclear cells; TEM, transepithelial migration; IBD, inflammatory bowel diseases; SGD, specific granule deficiency; LAD, leukocyte adhesion deficiency; MIP1 α , macrophage inflammatory protein 1 alpha; LTB-4, lipid leukotriene

B4; IECs, intestinal epithelial cells; MLCK, myosin light chain kinase; PARS, protease-activated receptors; DMs, Desmosomes; Dsg-2, desmoglein-2; Dsc-2, desmocollin-2; CAR, coxsackie and adenovirus receptor; CLMP, CAR-like protein; JAM, junctional adhesion molecules; SIRPa, signal regulatory protein alpha; CTX, thymocyte Xenopus; PI3K, phosphatidylinositol 3-kinase; EGFR, Epithelial growth factor receptor; AJs, adherens junctions; TJs, tight junctions; E-cadherin, epithelial cadherin; ICAM-1, intercellular adhesion molecule-1; EpCAM, epithelial cell adhesion molecule; TSP-1, thrombospondin-1; NO, nitric oxide; TGF- β , transforming growth factor beta; MMPs, matrix metalloproteases; EMT, epithelial-mesenchymal transition; CTLs, cytotoxic T lymphocytes; NF- κ B, nuclear factor kappa B; TIAM1, metastasis-inducing protein 1; sLea, sialyl Lewis A

Epithelial adhesion molecules play essential roles in regulating cellular function and maintaining mucosal tissue homeostasis. Some form epithelial junctional complexes to provide structural support for epithelial monolayers and act as a selectively permeable barrier separating luminal contents from the surrounding tissue. Others serve as docking structures for invading viruses and bacteria, while also regulating the immune response. They can either obstruct or serve as footholds for the immune cells recruited to mucosal surfaces. Currently, it is well appreciated that adhesion molecules collectively serve as environmental cue sensors and trigger signaling events to regulate epithelial function through their association with the cell cytoskeleton and various intracellular adapter proteins. Immune cells, particularly neutrophils (PMN) during transepithelial migration (TEM), can modulate adhesion molecule expression, conformation, and distribution, significantly impacting epithelial function and tissue homeostasis. This review discusses the roles of key intestinal epithelial adhesion molecules in regulating PMN trafficking and outlines the potential consequences on epithelial function.

Introduction

The gastrointestinal tract is constantly exposed to various toxins and pathogens; therefore, separating the luminal contents from the surrounding tissue is critical for proper tissue homeostasis and function maintenance. The epithelial cells lining the

gastrointestinal tract provide an essential protective barrier, selectively regulating fluid and solute exchange and keeping both the adaptive and the innate immune systems at check. Gastrointestinal disorders including inflammatory bowel diseases (IBD), acute gastritis, pancreatitis, and celiac disease are all phenotypically characterized by severely compromised epithelial barriers, luminal antigen leakage and increased inflammatory cell infiltrates.¹ Particularly IBD, encompassing ulcerative colitis and Crohn's disease, has been linked to transepithelial influx of polymorphonuclear leukocytes (PMNs) and their luminal space accumulation, which results in the formation of crypt abscesses. Patients with IBD suffer from chronic, active intestinal inflammation that waxes and wanes, where neutrophils are recruited to sites of inflammatory stimuli as a part of the normal gut inflammatory response. While the origin of the inflammation in IBD is yet unknown, altered epithelial permeability, invasion of commensal bacteria into the subepithelial space or lamina propria, and chemokine release by epithelial and resident immune cells, would be sufficient to drive massive neutrophil recruitment, and promote their accumulation in subepithelial spaces and the intestinal lumen. Studies showing that increased PMN numbers in patients stool² and epithelial crypts³ correlate with disease severity,⁴ imply that PMN-mediated inflammation may be the source of the compromised barrier function^{1,5} and epithelial injury² seen in IBD. However, it is yet unresolved issue whether PMN presence is a cause or an effect of the observed pathology, or whether it may be related/caused by other genetic or autoimmune defects often associated with these diseases. Patients with IBD also have significant alterations in the gut microbiota,⁶ which by itself may be a predisposing factor for IBD and initiate inflammatory responses and PMN recruitment.

While excessive PMN infiltrates in mucosal surfaces may be detrimental for tissue homeostasis, it is well known that PMNs

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also play a pivotal role in host defense against bacterial infections and contribute to inflammation resolution. In mucosal tissues, particularly in the gastrointestinal tract, rapid PMN mobilization toward the infection sites is critical for efficient pathogen clearance and successful adaptive immune response induction. Defective PMN recruitment, observed in patients with neutrophil-specific granule deficiency (SGD) and leukocyte adhesion deficiency (LAD), leads to frequent and severe bacterial infections and tissue damage.^{7,8}

PMN recruitment to mucosal tissues involves migration across vascular endothelial and epithelial monolayers. Both PMN trans-endothelial and transepithelial migration (TEM) have been extensively studied over the past few decades in a targeted effort to identify new anti-inflammatory therapies. While less defined than leukocyte migration across the vascular endothelium, multiple PMN-epithelial ligand pairs have been uncovered as key regulators of leukocyte TEM. Importantly, it is now well appreciated that during migration across the epithelial monolayer, PMNs interact with and modulate epithelial adhesion molecule function. PMNs can act directly or indirectly through the secretion of soluble mediators leading to signaling event initiation, which alters epithelial homeostasis.

In this review we will summarize the contributions of various basal, lateral, and apical (luminal) epithelial adhesion molecules to the regulation of epithelial barrier function, wound healing, and inflammation resolution during the different stages of PMN TEM (Table 1).

Neutrophil Recruitment to the Intestinal Mucosa

PMN recruitment to the intestinal mucosa and the subsequent luminal space accumulation is a sequential process, beginning with PMN extravasation from blood vessels, then migration through the interstitium, and finally with transepithelial migration (Fig. 1).

PMNs preferentially traverse the vascular endothelium at endothelial cell junctions.⁹ This requires PMN tethering/rolling along the endothelial surface,^{10,11} firm adhesion¹² and intraluminal crawling,^{13,14} which positions the PMNs at their preferred location. The regulatory mechanisms and specific molecules that mediate this process have been well defined and are elegantly detailed in a number of recent reviews.^{15,16}

Once the endothelial cell layer is crossed, PMNs migrate across the basement membrane and pericyte sheath,^{17,18} and continue navigating through the interstitium. The exact mechanisms underlying PMN migration through interstitial tissues are not well defined, but evidence suggests that this process is focal adhesion- and pericellular proteolysis- independent.¹⁵ PMN interstitial migration can be directed through secreted cytokines, such as CCL3 (MIP1α) and lipid leukotriene B4 (LTB-4), and requires actin polymerization and matrix metalloproteinase (MMP) activation.¹⁹ In contrast to transendothelial migration, PMNs migrating across epithelium must first engage the basolateral epithelial surface. From there, they navigate between the adjacent epithelial cells, where they encounter desmosomes, adherens, and tight junctions. While desmosomes primarily serve to maintain cell-cell adhesion and epithelial monolayer integrity,²⁰ tight and adherens junction components can transduce signals that regulate epithelial barrier function.^{21,22} Finally, after crossing the epithelial layer PMNs will emerge on the luminal (apical) surface, where they engage apically-expressed epithelial ligands.

PMN Binding to Basolaterally Expressed Epithelial Ligands and the Effects on Epithelial Function

PMN binding to the basolateral epithelial membrane is required for subsequent epithelial layer crossing. Unlike the vascular endothelium where PMNs use both CD11a/CD18 and CD11b/CD18 to adhere to and traverse the endothelial layer, in urinary, lung, and intestinal epithelium the initial PMN

Table 1: Epithelial adhesion molecules interact in trans to form epithelial junctional complexes that facilitate PMN migration and interaction with epithelial monolayers. (?-not determined)

Compartment	Epithelial adhesion molecule	Trans-interacting epithelial ligands	PMN counter-ligands
Basal	Fucosylated proteoglycans	?	CD11b/CD18 ³³
		Desmoglien-2	Caveolin-1 ¹⁴⁴
	Desmosomes	Desmocolin-2	Desmoglien-2 ⁴⁰
		E-cadherin	E-cadherin ^{145,146}
	Adherens junctions	Occludin	Occludin ¹⁴⁷
		Claudins	Claudins ^{77,144}
	Tight junctions	Tricellulin	Occludin [?] ¹⁴⁸
		JAM-A	JAM-A ^{149–151}
		JAM4	JAM4 ¹⁵²
		CAR	CAR ⁹⁵
		CD47	TSP-1 ¹⁰¹
		CD326 (EpCAM)	CD326, Claudin7, CD44v4-v7 ¹⁰³
		?	?
		?	CD11b/CD18 ^{11,31}
Apical	CD44	?	?
	CD54 (ICAM-1)	?	CD11b/CD18 ^{11,31}
CD11a/CD18 ¹⁵³	CD55 (DAF)	?	CD97 ¹³¹

Figure 1. Recruitment of circulating PMNs into the intestinal lumen. Upon release of a pro-inflammatory stimulus, circulating PMNs initiate contact with the endothelium resulting in PMN tethering/rolling (1), followed by firm adhesion and crawling (2), and terminating with migration across the endothelial monolayer, primarily at the junctional regions (3). After crossing the endothelium, PMNs navigate through the interstitium (4) until they arrive at the epithelium and engage in basolateral surface interactions (5). PMNs migrating across epithelium navigate between adjacent epithelial cells where they encounter desmosomes, adherens junctions, tight junctions, and other basolaterally expressed epithelial ligands, such as CD47 (6). After crossing the epithelial layer, PMNs emerge at the luminal (apical) surface where they engage apically expressed epithelial ligands, including ICAM-1, CD55, and CD44 (7). Finally, PMNs released into luminal spaces will apoptose and be cleared or, alternatively, accumulate in the crypt lumen to form abscesses, as observed under pathological conditions.

basolateral epithelial membrane attachment is almost exclusively dependent on CD11b/CD18.^{23,24} Intriguingly, while CD11b/CD18 has been shown to bind soluble factors such as fibrinogen,²⁵ iC3b,²⁶ elastase,²⁷ zymosan,²⁸ heparin sulfate,²⁹ and β -glucans,³⁰ as well as cell membrane proteins including ICAM-1,³¹ the basolaterally expressed epithelial ligands involved in PMN attachment are still largely unknown. Given the ability of CD11b/CD18 to bind heparin, cell surface proteoglycans decorated with heparin sulfate may serve as adhesive CD11b/CD18 ligands. However, these ligands have yet to be identified. Additionally, several reports demonstrated CD11b/CD18s ability to bind carbohydrates,³² thus cell surface proteoglycans decorated with sulfated fucose moieties are also proposed to be potential candidates for mediating CD11b/CD18-dependent neutrophil interactions with the basolateral epithelial surface. While addition of carbohydrates such as fucoidan, heparin sulfate, and N-acetyl-D-glucosamine significantly inhibited CD11b/CD18 binding to the basolateral epithelial surface^{33,34} the specific surface proteins carrying these carbohydrates have not yet been identified.

The identities of early PMN attachment and migration-mediating ligands at the basal/basolateral epithelial membrane remain elusive; however, it is well known that PMN basolateral surface binding during the early stages of TEM triggers changes in epithelial permeability. Loosening the epithelial barrier is essential for subsequent PMN epithelial layer crossing. For example, in intestinal epithelial cells (IECs) PMN contact alone, without entry into the epithelial paracellular space, was sufficient to increase epithelial permeability and enhance myosin light chain kinase (MLCK) phosphorylation. Notable, these initial changes were not associated with redistribution or loss of tight junction proteins.³⁵ Later work expanded these observations to show that

basolaterally adherent PMNs induced epithelial protease-activated receptors (PARs) 1 and 2 activation, leading to an MLCK-dependent increase in IEC permeability.³⁶

PMN Navigation of Inter-Epithelial Spaces and Consequent Effects on Epithelial Function

PMNs navigating the paracellular space between adjacent epithelial cells encounter a variety of lateral membrane complexes and adhesion receptors. Pathologic PMN TEM often results in relocalization, cleavage, and/or internalization of key junctional proteins, leading to prominent alterations in the epithelial function.

Desmosome signaling and function during PMN transepithelial migration

Desmosomes (DMs) are one of the first major obstacles PMNs encounter while migrating along the basolateral IEC membrane. DMs are critical epithelial intercellular junctions and appear as punctate structures forming cell-cell contacts in electron microscopy images. In the intestinal epithelium, DMs are transmembrane protein complexes formed from desmoglein-2 (Dsg-2) and desmocollin-2 (Dsc-2) and are anchored to keratin intermediate filaments via armadillo family proteins and desmoplakin (DSP).³⁷ Typically DMs are viewed as key structural elements; however, recent evidence links desmosomal cadherins to epithelial function-regulating signaling events. For example, Dsg-2 and Dsc-2 knockdown results in the mislocalization of zonula occludens-1, c-Src, and coxsackie and adenovirus receptor (CAR) at the cell-cell interface, leading to a perturbed blood-testis barrier.³⁸ These observations suggest that Dsg-2-dependent

cell-cell adhesion may play a role in regulating epithelial barrier function. In IBD, loss of Dsg-2, Dsc-2, and DSP expression correlated to significantly perturbed epithelial barrier function.³⁹ Similarly, in IECs, antibody-mediated inhibition of Dsg-2 homotypic binding resulted in loss of barrier function.⁴⁰ Desmosomal cadherins can be cleaved by MMPs.^{41,42} PMN granules contain a variety of MMPs that are released into the tissue during PMN transepithelial migration (TEM). Thus, PMN intestinal epithelium migration could potentially induce protease-dependent Dsg-2 cleavage, depleting cell-cell contacts.

In addition to forming epithelial junctional complexes, Dsg-2 is a high affinity receptor for Species B adenoviruses.⁴³ Moreover, virus binding by Dsg-2 triggered phosphatidylinositol 3-kinase (PI3K) and ERK1/2 activation, leading to the loss of epithelial cell characteristics, such as intercellular junctions, and the gain of mesenchymal cell properties. When virus binding is mimicked by addition of virus-produced particles or Dsg-2 antibody engagement, similar results were obtained, suggesting that direct Dsg-2 ligation can trigger signaling events to alter epithelial function.⁴³

Dsg-2 downregulation inhibited EGFR phosphorylation and EGFR receptor internalization, leading to decreased proliferation of intestinal epithelial cancer cells.⁴⁴ Furthermore, Dsg-2 has also been implicated in IEC apoptosis regulation.⁴⁵ Dsg-2 loss also leads to expression changes in Dsc-2, its desmosomal partner, which has been shown to promote epithelial cell proliferation and tumor growth in vivo through Akt/ β -catenin signaling activation.⁴⁶

These results indicate that under inflammatory conditions the PMNs associated with epithelial monolayers modulate Dsg-2/Dsc-2 expression and function, which can subsequently affect epithelial barrier function, proliferation, and apoptosis.

The desmosomal armadillo proteins plakoglobin and plakophilin, as well as the plakin family member, desmoplakin, reside within the desmosomes and mechanically link desmosomal cadherins to intermediate filaments and the cytoskeleton.^{20,47} In addition to desmosomal functions, these adapter proteins regulate actin organization, protein synthesis, growth control, and cell proliferation.⁴⁸ Intriguingly, these adaptor proteins' localization and function during PMN TEM is still unclear and should be investigated in the future.

Adherens junction signaling and function during PMN transepithelial migration

As PMNs migrate basolaterally toward the apical epithelial membrane they encounter epithelial apical junctional complexes, comprised of adherens junctions (AJs) and tight junctions (TJs). AJs and TJs tightly bind adjacent epithelial cells, forming a selective barrier that separates luminal contents from the surrounding tissue. They share structural similarities and have some overlapping functions; however, there are also some distinct cellular functions.

AJs are formed through homophilic interactions between extracellular epithelial cadherin (E-cadherin) domains and function as cell-cell adhesion mediators. The E-cadherin cytoplasmic tail associates with an array of intracellular proteins including the

catenin family members, p120-catenin and β -catenin. These adapter proteins link cell-cell adhesions to the actin-myosin network, while also playing a crucial role in promoting cell adhesions through E-cadherin stabilization at the cell membrane. As such, loss of p120-catenin concomitantly leads to E-cadherin loss.⁴⁹ p120 also plays an essential role in intestinal barrier function. Its deletion resulted in aberrant inflammation and amplified neutrophil recruitment in mouse intestines during bacterial colonization.⁵⁰ E-cadherin surface expression and/or localization alterations alone can modulate vesicular transport and signal transduction to the nucleus, which alters gene expression.^{51,52} Furthermore, AJ assembly is required for tight junctional complex formation and alterations in epithelial cadherin expression results in epithelial paracellular barrier changes.^{53,54}

PMN migration across various epithelia has been shown to deplete both E-cadherin and β -catenin from the cell membrane. For example, during PMN penetration of airway epithelium under inflammatory conditions, the E-cadherin extracellular domain is cleaved by PMN elastase, instigating AJ destabilization.^{55,56} In gingival epithelium, A. actinomycetemcomitans-induced PMN infiltration resulted in a p38 MAPK-dependent decrease in E-cadherin level.⁵⁷ Similarly, in intestinal epithelium, E-cadherin and β -catenin focal loss was observed immediately adjacent to clusters of transmigrating neutrophils, resulting in disruption of junctional complexes.⁵⁵

Neutrophils infiltrating epithelial monolayers secrete TGF- β ⁵⁸ and serve as a dominant source of nitric oxide (NO)⁵⁹ and MMPs including MMP9.⁶⁰ In addition to acting as a potent chemoattractant for PMNs, TGF- β also induces cadherin contact disruption.⁶¹ Similarly, both NO and MMP9 were shown to degrade/cleave membrane-bound E-cadherin at cell junctions, resulting in β -catenin nuclear translocation and β -catenin/LEF-1 complex formation.^{62,63} Since disruption of AJs can alter TJ organization, it is not surprising that PMN-induced E-cadherin loss was associated with impaired epithelial barrier function.⁵⁴ On the other hand, PMN-induced activation of LEF-1 gene transcription led to enhanced epithelial cell proliferation, which may promote reparative mechanism initiation within the epithelium.⁶⁴

Importantly, E-cadherin plays a key role in epithelial cell polarity maintenance and acts as a potent tumor suppressor by firmly adhering adjacent epithelial cells, preventing their migration and invasion of other tissues.^{65,66} E-cadherin loss is considered a hallmark of epithelial-mesenchymal transition (EMT). EMT is a mechanism intended to form mesenchymal cells in injured tissues; however, it can initiate invasive and metastatic behavior in epithelial cancers. As such, E-cadherin loss facilitated the transition of lung cancer cells into the mesenchymal type and increased their motility and invasiveness in an MMP2-dependent manner.⁶⁷ Given E-cadherin's role in EMT and tumor suppression, PMN-induced E-cadherin depletion within epithelial tumors is likely to contribute to tumor metastasis. Indeed, PMN elastase-mediated E-cadherin degradation induced dyshesion and increased the migratory capacity of pancreatic tumor cells.⁶⁸

Finally, E-cadherin serves as a receptor for *Listeria monocytogenes* and is required for this intracellular pathogen's entry into

ECs.⁶⁹ It also serves as an $\alpha\text{E}\beta 7$ integrin ligand on CD103-expressing cytotoxic T lymphocytes (CTLs), which play critical roles in antitumor immune response. The $\alpha\text{E}\beta 7$ integrin interaction with E-cadherin promotes antitumor CTL activity by triggering lytic granule polarization and exocytosis.⁷⁰ In these cases, PMN-mediated depletion of surface E-cadherin may affect pathogen entry and promote neoplastic transformation.

Tight junction signaling and function during PMN transepithelial migration

Tight junctions (TJs) are positioned at the most apical lateral surface between adjacent epithelial cells; therefore, migrating PMNs encounter TJ proteins late during TEM, immediately prior to their arrival at the apical epithelial surface. TJs are formed between transmembrane proteins localized to adhesive barrier strands and include claudins, occludins, tricellulin, and immunoglobulin superfamily members. They also include related molecules such as coxsackie and adenovirus receptor (CAR), the junctional adhesion molecules JAM-A and JAM4, and CAR-like protein (CLMP).

Localization of TJ proteins to lateral cell membranes makes them attractive candidate receptors for migrating leukocytes; however, their role in PMN TEM is still not well defined. JAM-A, one of the most studied tight junction-associated cortical thymocyte Xenopus (CTX) proteins, has been shown to mediate PMN migration across endothelial monolayers *in vivo* in various inflammation models.^{71,72} However, in the intestinal epithelium, downregulation/antibody-mediated inhibition of JAM-A's barrier forming properties had no significant effect on PMN TEM.⁷³ Similarly, inhibition of CAR heterophilic interactions with its PMN counter-ligand, JAML, had only minor effects on PMN TEM. The inability to significantly impact PMN TEM by inhibition of these interactions was attributed to JAML shedding during PMN TEM.⁷⁴ The PMN TEM-regulating roles of other important epithelial TJ constituents, including claudins and tricellulin, are also unclear. Interestingly, PMN migration across endothelial monolayers has been shown to preferentially occur at tricellular junctions.⁹ Whether this is also true for PMN TEM remains to be determined. Given the exclusive localization of tricellulin to epithelial tricellular junctions and its role in controlling paracellular macromolecule movement at tricellular epithelial tight junctions,⁷⁵ it is reasonable to speculate that it could contribute to PMN TEM regulation. Emerging evidence suggests that occludin may modulate PMN TEM, as overexpression of occludin with a mutated extracellular domain was reported to inhibit PMN migration across epithelial monolayers.⁷⁶ However, it is still unclear whether migrating PMNs can actively engage occludin during TEM or if it is a secondary effect of disturbed epithelial monolayer stability resulting from diminished occludin trans interactions.

While TJ proteins' contributions to PMN epithelial monolayer migration are not well defined, TJ proteins perform many distinct and overlapping functions to regulate epithelial homeostasis through actin cytoskeleton interactions and signaling complex formation. For example, claudins constitute both paracellular barriers and pores, thereby playing a key role in

determining the epithelial monolayer permeability properties.^{77,78} While evidence conflicts regarding occludin's role in epithelial permeability regulation, occludin is important for TJ formation, TJ stability maintenance,⁷⁹ claudin expression regulation,⁸⁰ caspase-mediated apoptosis activation,⁸¹ and apoptotic cell extrusion from the epithelial monolayer,⁸² as well as regulating epithelial cell motility.⁸³ While JAM-A does not directly contribute to TJ formation in epithelial cells, it can regulate key cellular functions including paracellular permeability,⁸⁴ cell polarity,⁸⁵ claudin expression,⁸⁶ cell proliferation and motility.^{87,88} For example, JAM-A can regulate apical actomyosin ring contractions through RhoA activation.⁸⁴ In turn, RhoA can activate Rho-kinase, leading to claudin-5 and occludin phosphorylation.⁸⁹ Induction of either of these events would result in altered epithelial permeability.

Taken together, it is not surprising that TJ assembly alterations significantly compromise epithelial barrier function, which is a hallmark of several gastrointestinal disorders including inflammatory bowel disease (IBD).^{7,8} Another characteristic of IBD is high levels of PMN accumulation in intestinal mucosal tissues.⁴ Importantly, PMN infiltration of mucosal epithelia significantly impacted TJ protein expression and localization. As such, in human IBD patient tissue collected from PMN accumulation regions, a significant decrease in occludin, claudin-1, ZO-1, and JAM-A expression was observed.⁹⁰ Similar observations were made in an *in vitro* setup simulating PMN migration across epithelial monolayers. As shown in Fig. 2 (unpublished observations) PMN migration across cultured human epithelial cells grown on permeable supports led to significant downregulation/internalization of key TJ and adherence junction components, occludin (left panel) and E-Cadherin (right panel). Therefore, PMN migration across epithelial monolayers can modulate protein expression/distribution at the epithelial junctions and significantly impact tissue homeostasis. Indeed, *in vitro* PMNs migrating across epithelial monolayers caused a transient decrease in epithelial barrier function that is TJ-independent in the early phase and TJ-dependent in the later phase.³⁵ It is also well known that modulating expression of specific claudins can lead to either tighter or leakier epithelial phenotypes.⁷⁷ Since both JAM-A and occludin can regulate various claudin expression levels^{80,86} and PMN can modulate JAM-A/occludin function, migrating PMNs may lead to the preferential expression of leaky claudins over tight ones, exerting long-term effects on epithelial barrier function.

Epithelial healing and restitution is an integral part of mucosal homeostasis. This process, involving cell migration and/or proliferation, is associated with the TJ protein JAM-A. JAM-A associates with the scaffold protein afadin and the guanine nucleotide exchange factor PDZ-GEF2 to activate Rap1a, stabilize $\beta 1$ integrins, and enhance cell migration.⁹¹ However, JAM-A has been shown to restrict IEC proliferation by inhibiting Akt-dependent β -catenin activation.⁸⁷ Consistently, JAM-A-deficient epithelia exhibit hyperproliferative phenotypes.⁸⁶ Since PMNs can alter JAM-A expression, PMN-epithelial monolayer association may affect epithelial proliferative response and cell migration, thereby impacting epithelial repair.

Figure 2. PMNs migrating across epithelial monolayers in vitro significantly alter TJ protein expression and localization. PMNs (red) migrating across epithelial monolayers grown on permeable supports in a physiologically relevant basolateral to apical direction, locally downregulate/trigger internalization of junctional proteins including occludin (green, left panel) and E-cadherin (green, right panel). Both panels are projections of images acquired in series in Z-direction (apical to basolateral). Left panel depicts PMN during initial contact with the basolateral surface of the epithelial monolayer, prior to TEM (early TEM, indicated by the white arrow), and PMN emerging at the apical epithelial membrane (late TEM, indicated by the white arrow). During early phases of PMN TEM, occludin expression patterns are not yet perturbed, however as PMN migrate across the epithelium loss of occludin can be observed. Similarly, internalization and loss of E-cadherin is observed during late phases of PMN TEM (right panel). Thus, migrating PMNs can significantly impact tissue homeostasis through junctional protein expression/distribution modulation. The bar is 20 μ m.

JAM-A has also been shown to participate in homophilic interactions with itself. These interactions have been shown to be critical for epithelial permeability regulation.⁸⁴ Recent studies in epithelial cells demonstrate that JAM-A forms homodimers between cells.⁹² These interactions proved important in regulating epithelial permeability through Rap2 activity modulation. The consequences of these interactions on epithelial function during PMN TEM remain to be determined; however, this could serve as a potential mechanism for PMN-dependent barrier impairment during TEM.

CAR is another TJ component that has been implicated in regulating epithelial cell adhesion, permeability, junctional stability, and wound healing.^{74,93,94} Similar to JAM-A, CAR can engage in homophilic interactions⁹⁵; however, it also binds JAML, a JAM-like protein expressed exclusively on PMNs, monocytes, and $\gamma\delta$ T-cells,^{74,96} with high affinity. While these observations marked the CAR-JAML ligand pair as an attractive candidate for PMN trafficking regulation, a recent study found that inhibiting these interactions only had a minor effect on PMN TEM. Instead, PMN shed JAML during TEM and the binding of soluble JAML to CAR resulted in faulty epithelial resealing and significantly impaired mucosal wound healing.⁷⁴ These findings defined CAR as a key regulator of intestinal homeostasis and identified a novel PMN infiltration mechanism that may affect both epithelial barrier and wound healing.

Currently, it is not entirely clear how CAR regulates junctional stability and epithelial wound healing; however, it has been recently proposed to modulate E-cadherin dynamics and recruitment to cell-cell contacts.⁹⁴ Specifically, PKC δ -mediated C-terminal CAR phosphorylation stabilizes E-cadherin at the cell junctions,⁹⁴ likely resulting in reduced migratory and proliferative phenotypes.

These results demonstrate that TJ proteins perform numerous, intertwined and complex roles to regulate epithelial function. By modulating their expression and activity, PMNs may significantly impact epithelial homeostasis.

Non-junctional epithelial adhesion molecules, signaling and function

In addition to the adhesion molecules involved in epithelial junctional complex formation, several other PMN TEM-associated candidates have been identified for their ability to both regulate PMN TEM and trigger epithelial function modulation signaling events. One example is CD47, an immunoglobulin superfamily member⁹⁷ expressed both on the basolateral epithelial surface and on neutrophils.⁹⁸ CD47 has been suggested to fine tune the PMN migration rate, ensuring their timely arrival to inflammatory sites in vivo. As such, enhanced PMN TEM has been shown to correlate with increased CD47 expression on epithelial cells. Antibody-mediated inhibition of CD47 or its PMN

counter-ligand, signal regulatory protein α (SIRP α),⁹⁹ significantly delayed neutrophil TEM in vitro.¹⁰⁰ Intriguingly, CD47 ligation with haematopoietic cells can induce intracellular signaling that results in cell activation or cell death depending on the exact context. For a review see ref.¹⁰⁰ Similarly, thrombospondin-1 (TSP-1) ligation of CD47 triggers apoptosis in endothelial cells¹⁰¹ and fibroblasts.¹⁰² Therefore, it is tempting to speculate that CD47 ligation in epithelial cells during PMN TEM may trigger CD47-dependent intracellular signaling events involved in epithelial homeostasis regulation.

EpCAM is another adhesion molecule expressed at the basolateral membrane of non-inflamed human epithelial cells; however, it is also considered a cancer marker because its expression is markedly increased in various carcinomas.¹⁰³ In addition to mediating cell-cell adhesion, EpCAM plays a role in regulating epithelial cell proliferation, migration, and transduction of mitogenic signals.¹⁰⁴ In inflammation, its extracellular and intracellular domains are sequentially cleaved by tumor necrosis factor α -converting enzyme (TACE/ADAM17) and gamma-secretase complex-containing presenilin 2, respectively. The released intracellular EpCAM domain binds the transcriptional regulators β -catenin and Lef to form a large nuclear complex that stimulates transcription of proliferative and oncogenic genes including c-Myc and cyclins.¹⁰⁵ Interestingly, cell-to-cell contact has been identified as an initial trigger for EpCAM activation¹⁰⁶; therefore, one can hypothesize that PMNs migrating across epithelial monolayers may engage EpCAM and trigger EpCAM-dependent event activation. However, this has yet to be studied.

Apically Expressed Adhesion Molecule Signaling and Function During PMN Transepithelial Migration

After navigating the paracellular space between adjacent epithelial cells, PMNs arrive at the apical (luminal) surface. Extensive efforts have been dedicated to identifying the key players that mediate PMN TEM and to defining the effects of PMN migration on epithelial function. However, while significant progress has been made in recent years, our understanding of this process is still limited. Even less is known about PMN interaction with apical IEC ligands or the potential effects these interactions have on epithelial homeostasis. In a number of inflammatory disorders, including acute lung injury,¹⁰⁷ cystic fibrosis,¹⁰⁸ and IBD,³ PMNs accumulate in luminal spaces and remain in intimate contact with apical epithelial cells. Apically retained PMNs can remain in contact with the epithelium for extended periods, as PMN activation/TEM delays their apoptosis.¹⁰⁹⁻¹¹¹ Therefore, apically expressed epithelial adhesion molecule engagement by PMNs may impact epithelial function. PMN interactions with luminal epithelial membranes have recently come into focus with the identification of several apically expressed epithelial PMN ligands.

Specifically, CD54 (ICAM-1),¹¹² CD55 (DAF),¹¹³ and CD44 expression¹¹⁴ has been shown to increase under inflammatory conditions. Importantly, these adhesive receptors have been

implicated in mediating PMN interactions with the apical epithelial membrane. They also act as signaling molecules, capable of triggering intracellular signaling events involved in epithelial function regulation.

ICAM-1 is a known PMN CD11b/CD18 ligand.³¹ It plays a critical role in neutrophil adhesion to and migration across the endothelium.^{11,115} Moreover, it is a signal-transduction molecule that links leukocyte-endothelial cell interactions with endothelial permeability regulation. When adherent leukocytes engage ICAM-1, it signals cell cytoskeleton and interendothelial junction reorganization to enhance solute exchange and promote leukocyte migration.^{116,117} ICAM-1 is also significantly upregulated and localized at the apical surface in various epithelia under inflammation conditions.¹¹⁸⁻¹²² Since PMN migration across epithelial monolayers occurs in the basolateral to apical direction, this expression pattern invalidates a role for ICAM-1 in directly mediating initial PMN adhesion and TEM.¹¹⁸ Intriguingly, while ICAM-1 plays no apparent role in PMN adhesion to the basolateral epithelial surface,¹¹⁸ we recently reported that it might have indirect effects on PMN TEM through regulating epithelial permeability.¹²³ As such, ICAM-1 ligation by transmigrated PMNs triggered MLCK-dependent cell contraction, enhancing intestinal epithelial permeability and promoting PMN TEM.¹²³ ICAM-1 expression has also been linked to epithelial wound healing¹²⁴; however, the mechanisms involved are still under investigation. One proposed mechanism implicated ICAM-1 in mediating $\gamma\delta$ T cell accumulation at injured corneal epithelial sites, allowing the T-cell to exert beneficial wound healing effects.¹²⁵ Alternatively, ICAM-1 has been implicated in regulating cell motility¹²⁶ and activating key cell proliferation signaling molecules. Therefore, it is tempting to speculate that when ICAM-1 is engaged by transmigrated PMNs in the intestinal lumen it may trigger reparative mechanism initiation.

The balance between PMN mobilization, retention, and clearance at insult sites is essential for host defense, efficient inflammation resolution, barrier function restoration, and tissue repair initiation. Therefore, it is not surprising that some adhesion receptors, such as ICAM-1, serve to retain PMNs at the apical membrane, while others, including CD55 and CD54, promote PMN clearance by mediating their detachment into the intestinal lumen.

CD55 is known to inhibit complement activation by interfering with C3 and C5 convertase function.¹²⁷ However, it also serves as a ligand for pathogens such as adhering *Escherichia coli*¹²⁸ and for PMNs migrating across epithelial monolayers.¹²⁹ Importantly, CD55 binding has been shown to induce epithelial signaling, resulting in NF- κ B nuclear translocation and MAPK pathway activation.¹³⁰ Furthermore, bacterial CD55 binding-induced upregulation of MICA, an epithelial MHC-I homolog, leads to pro-resolution $\gamma\delta$ T-cell recruitment.¹²⁸ While PMN binding of CD55 leads to rapid detachment from the epithelial membrane, the signaling events involved are still not clear. CD97 is a well-characterized, non-complement ligand for CD55 that is expressed on PMN. CD97 has been suggested to play a role in PMN recruitment to the inflamed intestinal mucosa,¹³¹ however, it is not clear whether these effects were specific to its

interactions with epithelial CD55. Indeed, it is unknown whether specific CD97-CD55 interactions play a role in neutrophil trafficking across epithelial monolayers. It also remains to be established whether PMN CD55 binding can trigger intracellular epithelial signaling. Similar to CD55, epithelial expressed CD44 facilitates PMN detachment during TEM.¹³² CD44 also mediates signaling events that regulate epithelial cell growth, survival, differentiation, proliferation, and motility through association with key signaling molecules including Src, Rho GTPase, Rho kinase, metastasis-inducing protein 1 (TIAM1), VAV₂, the proto-oncogene protein-tyrosine kinases LCK and FYN, and PKC. For a review see reference.¹³³ In vitro, CD44 interactions with hyaluronan, collagen, laminin, and fibronectin,^{134,135} as well as RhoA binding promote epithelial cell migration and tumor metastasis.¹³⁶ Similar to ICAM-1, CD44 regulates the actin cytoskeleton through associations with the ERM proteins ezrin, radixin and moesin.¹³⁷ CD44 can also recruit MMP9 and MMP7 to the cell surface. Through the MMP activity it can influence epithelial junctional complex regulation, epithelial tumor invasiveness,¹³⁸ and apoptosis.¹³⁹

Recent work suggested that a CD44 splice variant, CD44v6, promotes PMN detachment from apical intestinal epithelial surfaces after completion of TEM.¹³² Furthermore, this study demonstrated that PMN TEM resulted in generation of soluble CD44 (sCD44), likely through MMP-dependent CD44 extracellular domain cleavage. Intriguingly, a follow-up work determined that PMN binding to CD44v6 is mediated by protein-specific O-glycosylation with sialyl Lewis A (sLe^a), and inhibition of sLe^a prevented the cleavage of CD44V6.¹⁴⁰ sCD44 has been successfully used to neutralize CD44 function and inhibit tumor cell invasion and proliferation in cancer cells.^{139,141} Importantly, extracellular CD44 domain release leads to presenilin-1/ γ -secretase-mediated cleavage of the CD44 intracellular domain.¹⁴² These intracellular fragments then translocate to the nucleus to stimulate phorbol ester response element and transcription of its target genes.¹⁴³ Given these observations, PMNs migrating across epithelial layers can engage and modulate expression, localization, and activity of apically expressed epithelial ligands to impact the regulation of both PMN recruitment and mucosal tissue function and homeostasis.

Concluding Remarks

Epithelial adhesion molecules play essential roles in regulating cellular function and tissue homeostasis. Epithelial junctional

complex-forming molecules have been extensively studied and their roles in cell monolayer structural support, as invading virus and bacteria receptors, and as signals for epithelial function regulation are well defined. Thus it is not surprising that they serve as attractive target molecule for new antibody-based therapies. Indeed, numerous epithelial adhesion molecules including desmosomal cadherins, claudins, CAM family molecules, such as EpCAM (CD326) are currently investigated as potential diagnostic and therapeutic anti-cancer targets. Furthermore, it is becoming apparent that epithelial adhesion molecules can also modulate immune responses by participating in leukocyte trafficking regulation; however, interacting leukocytes can also modulate their function, leading to epithelial response alterations. The function of non-junctional adhesion molecules expressed on either the basolateral or apical epithelial membranes is less defined; however, increasing evidence suggests that they may fulfill important roles in regulating epithelial function by sensing environmental cues and transducing outside-in signaling when engaged by interacting immune cells. As has been outlined in this review the complex nature of PMN interactions with epithelial adhesion molecules during PMN infiltration of mucosal surfaces may lead to both pathological and beneficial outcomes. As a result simply inhibiting neutrophil trafficking may not achieve the ultimate goal of resolving inflammation and improving patient's symptoms. Thus, one of the biggest challenges for future work remains the identification of new epithelial ligands involved in these responses, and defining the multiple functions fulfilled by individual adhesion molecules. Better understanding the roles epithelial adhesion molecules play in leukocyte trafficking and in signal transduction that regulates epithelial cell function will assist in designing specific therapeutic approaches to decrease the pathologic symptoms without impeding the potential beneficial outcomes. This is a rich and evolving field that promises exciting outcomes for future exploration.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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