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Disassembly of endothelial and epithelial junctions during leukocyte transmigration

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1. ABSTRACT

Leukocyte migration occurs as a response to inflammatory signals and is an efficient host defense mechanism against invading pathogens. This innate defense response includes transendothelial and transepithelial migration of leukocytes to facilitate clearance of inflammatory stimuli. The endothelium lines the vascular system and forms the first barrier for leukocytes as they migrate out of the bloodstream. The epithelium largely separates organs from the external environment and forms a second barrier for leukocytes. These cellular barriers are comprised of complex intercellular junctions of different molecular composition. However, for barrier function to be maintained, these specialized intercellular junctions must not be destroyed during transmigration. Innate immune cells including monocytes, neutrophils and dendritic cells are all capable of a highly regulated transmigration response in order to accomplish their different functions. These cells exploit many common adhesive and signaling cascades to traverse cellular junctions. However, there are unique features of each type of leukocyte and barrier that determine specificity of the response. This review will focus on highlighting the mechanisms that leukocytes exploit to open these junctions.

2. INTRODUCTION

Leukocyte migration across endothelial and epithelial cell layers (referred to as transmigration) is a central component of the (innate) immune response. Inflammatory signals induce the expression of endothelial cell proteins that attract circulating leukocytes which are then captured on the endothelial surface where they roll until they firmly adhere. The adhesion of leukocytes to endothelial apical adhesion molecules is the first step of leukocyte activation that leads to extravasation, a process termed diapedesis (1). This process is still not fully understood but requires the coordinate function of adhesion molecules, the cytoskeleton and signaling molecules. Different adhesion molecules are major components of cellular junctions that seal the paracellular space and therefore form a barrier for leukocytes. During diapedesis, endothelial junctions are reversibly disassembled to allow leukocyte passage without vascular disruption (2). Once outside of the blood vessel, the activated leukocytes migrate to the site of inflammatory stimulus, respond and participate in tissue remodeling and wound healing. A further step during many types of inflammatory conditions involves transepithelial migration of leukocytes. The major function of epithelial cells is to separate different body

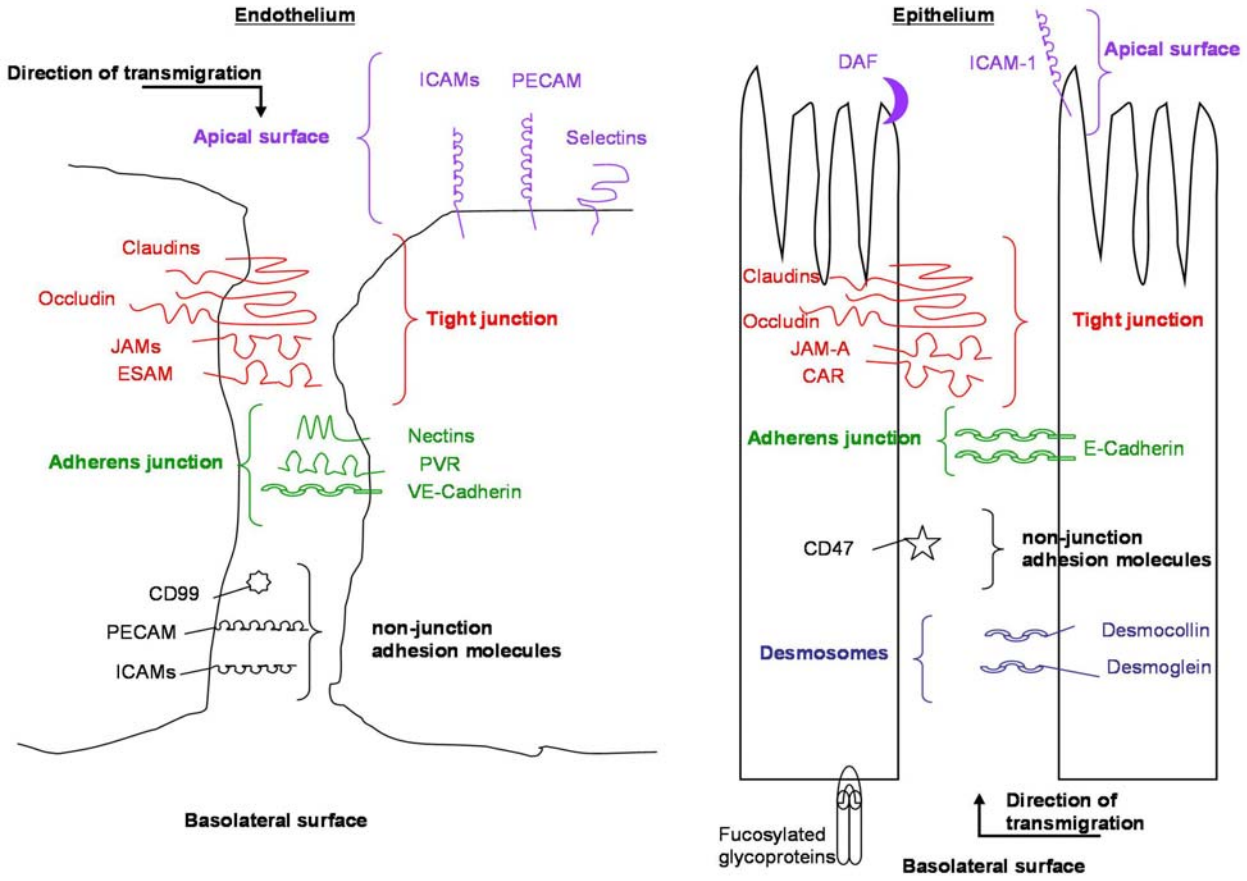

Figure 1. Endothelial and epithelial adhesion molecules. The junctions in the paracellular space are built mostly by homophilic interactions of the shown adhesion molecules. When junctions open up during transmigration, these adhesion molecules are engaged in both homophilic and heterophilic interactions with adhesion molecules on the leukocyte surface. The direction of leukocyte transmigration is indicated by arrows. DAF, decay accelerating factor. AJC, apical junction complex.

compartments or to protect from the highly contaminated external environment. In this respect, for example, the epithelium of the lung, urinary tract and the intestines serves as an effective barrier against a sea of potentially pathogenic microorganisms. In these organs neutrophils migrate across the epithelium to combat invading microorganisms. Thus, epithelial junctions form a barrier for both pathogens in the exterior and leukocytes in the interior.

During normal immune surveillance, there is a continuous, low level of transmigrating leukocytes that reversibly disassemble endothelial and epithelial junctions without significant effects on cell barrier. However, during major inflammatory responses as they occur, for instance, in idiopathic inflammatory bowel disease (IBD), massive transmigration of neutrophils destroys intercellular junctions resulting in a significantly compromised epithelial barrier (3,4). This disruption of intercellular junctions is accompanied by altered signal transduction pathways leading to changes in tissue morphology and increased endothelial/epithelial permeability. Different leukocytes exploit different mechanisms to open up endothelial or epithelial junctions that require the concerted action of different adhesion and signaling molecules. These different mechanisms will be highlighted in this review.

Although there are similarities in junctional components and signaling events between endothelial and epithelial cells, there are unique features defining each (5-9). Common as well as different junctional adhesion molecules in endothelial and epithelial cells that participate in the transmigration process are depicted in Figure 1.

Since leukocyte transendothelial and transepithelial migration has recently been reviewed in detail elsewhere (2,10-13), we will focus in this review on the comparison of different adhesion and signaling mechanisms that are exploited by different leukocytes to (reversibly) open up endothelial and epithelial junctions during inflammatory responses.

3. THE ENDOTHELIUM

3.1. Endothelial junctions

The endothelium lines the vascular system in the body and separates the bloodstream from tissues. The endothelium regulates exchange of solutes and fluid

between blood and tissue and controls the rolling and adhesion of leukocytes through the intricate involvement of inflammatory signals and adhesion molecules (2). There are significant site-specific differences in endothelia within different tissues of the body such as in the brain and the testis. However, a detailed description of these specialized endothelial barriers is beyond the scope of this review and can be found elsewhere (14-17). Here, we will restrict the description of endothelial junctions to the basic components that are crucial to leukocyte extravasation from the blood into tissues. An intact endothelial monolayer is maintained by the connection of each single cell to its neighboring cells through two major types of intercellular junctions: tight (TJ) and adherens (AJ) junctions. Due to irregular cell shapes within the endothelial cell layer, bi-, tri- and multicellular borders exist that display different junction strengths and are preferentially selected by different leukocytes for transmigration (18). These intercellular junctions are built of a complex network of transmembrane adhesion proteins which, through their cytoplasmic tails, associate with numerous cytoplasmic and cytoskeletal proteins (19). These associations allow the delivery of signals into the cell that can eventually result in an altered junction composition and barrier function, the mechanisms of which we will discuss later in this review. A schematic illustration of adhesion molecules that build different types of endothelial junctions is shown in figure 1A. Major transmembrane components in the endothelial tight junctions are claudins, occludin, junctional adhesion molecules (JAMs) and endothelial selective adhesion molecule (ESAM). Major transmembrane proteins comprising endothelial adherens junctions include vascular endothelial (VE)-cadherin, nectins and the nectin-related poliovirus receptor (PVR) (2,8). The cellular localization of the above intercellular junction proteins is highly specific since many other important adhesion molecules are located throughout the lateral membrane and the apical surface that are not confined to either tight or adherens junction. Examples of these types of proteins include platelet endothelial cellular adhesion molecule (PECAM), intercellular adhesion molecules (ICAMs) and CD99 (2).

A key function of the tight junction is to regulate paracellular permeability, or leakiness between cells (20,21). Permeability is directly regulated by different combinations of transmembrane TJ molecules that form characteristic sub-apical strands varying from a complex organization in the highly impermeable blood brain barrier to a much more loosely organized meshwork in the vascular endothelia of other tissues. In these tissues with more permeable TJs, adherens junctions play a more dominant role in regulating endothelial permeability (22-24). The development of junctional tightness and vascular permeability during angiogenesis is affected by the microenvironment surrounding the endothelium in that vascular bed. This was demonstrated in a study by Stewart and Wiley by transplanting gut tissue into the brain (25). In this tissue, neovascularization occurred by brain endothelial cells which displayed characteristics of gut endothelium. However, when non-neural endothelial cells were cocultured with astrocytes or in the presence of astrocyte-conditioned medium, the same endothelial cells developed tight junctions with characteristics similar to that of the impermeable blood-brain-barrier (26-28). These studies suggest that the tissue-specific microenvironment plays a profound role in defining the endothelial permeability.

Another critical function of the vascular endothelial intercellular junctions is to control the extravasation of leukocytes. Leukocyte transendothelial migration occurs from the lumenal or apical surface of the endothelium, across intercellular junctions, and along the basolateral site of the endothelium to finally reach the basement membrane. The extravasation of leukocytes is initiated by the induction of adhesion molecules at the apical endothelial surface to capture leukocytes. A key player in the arrest of all circulating leukocytes from the blood stream is ICAM-1 which serves as a ligand for the leukocyte integrin LFA-1 (Figure 2) (29,30). After firm arrest, leukocytes extend pseudopods across endothelial intercellular junctions which results in a reversible opening of the junctions and a reversible increase in paracellular permeability. This event requires extensive remodeling of the cytoskeleton and several sequential adhesive steps in which many different adhesion molecules are involved depending on the transmigrating type of leukocyte.

3.2. Mechanisms of junctional opening during leukocyte transendothelial migration

Leukocyte extravasation into inflamed tissues occurs mostly in post-capillary venules. The initial capturing and rolling of leukocytes on the endothelial surface is mediated by selectins (Figure 2) (31,32). These adhesion proteins bind to glycosylated leukocyte ligands in a transient fashion allowing for rolling on the endothelial surface until they become firmly attached. Proteins responsible for firm attachment of leukocytes to the endothelium include members of the β2-integrin family such as LFA-1 (CD11a/CD18). This integrin is expressed on the surface of all leukocytes (33,34) and binds to members of the immunoglobulin superfamily (IgSF) of adhesion molecules, namely ICAM-1 and 2 and VCAM (35). However, dendritic cells (DC), specialized antigen-presenting cells (36), use a DC-specific lectin (DC-SIGN) to adhere to endothelial cells. Interactions of DC-SIGN with ICAM-2 facilitate tethering and rolling under shear flow and regulate chemokine-induced transmigration of DCs across resting as well as activated endothelium (37). ICAMs are expressed on both the apical and lateral membrane of vascular endothelium and may thus be involved not only in adhesion but also transmigration of leukocytes. VCAM is only expressed on the apical membrane to trigger adhesion of leukocytes (38). Neutrophil LFA-1 has been shown to redistribute and cluster with endothelial ICAM-1 in a ring-like structure at sites of neutrophil-endothelial contact when neutrophils have entered the paracellular space indicating the importance of both of these molecules during transmigration (39). It has also been shown that junctional adhesion molecule (JAM)-A, a member of the IgSF and TJ molecule in both endothelium and epithelium (40), colocalized with this ring-like structure suggesting that JAM-A may play some role in the formation of this structure. In a recent study, ICAM-1 enriched cup-like

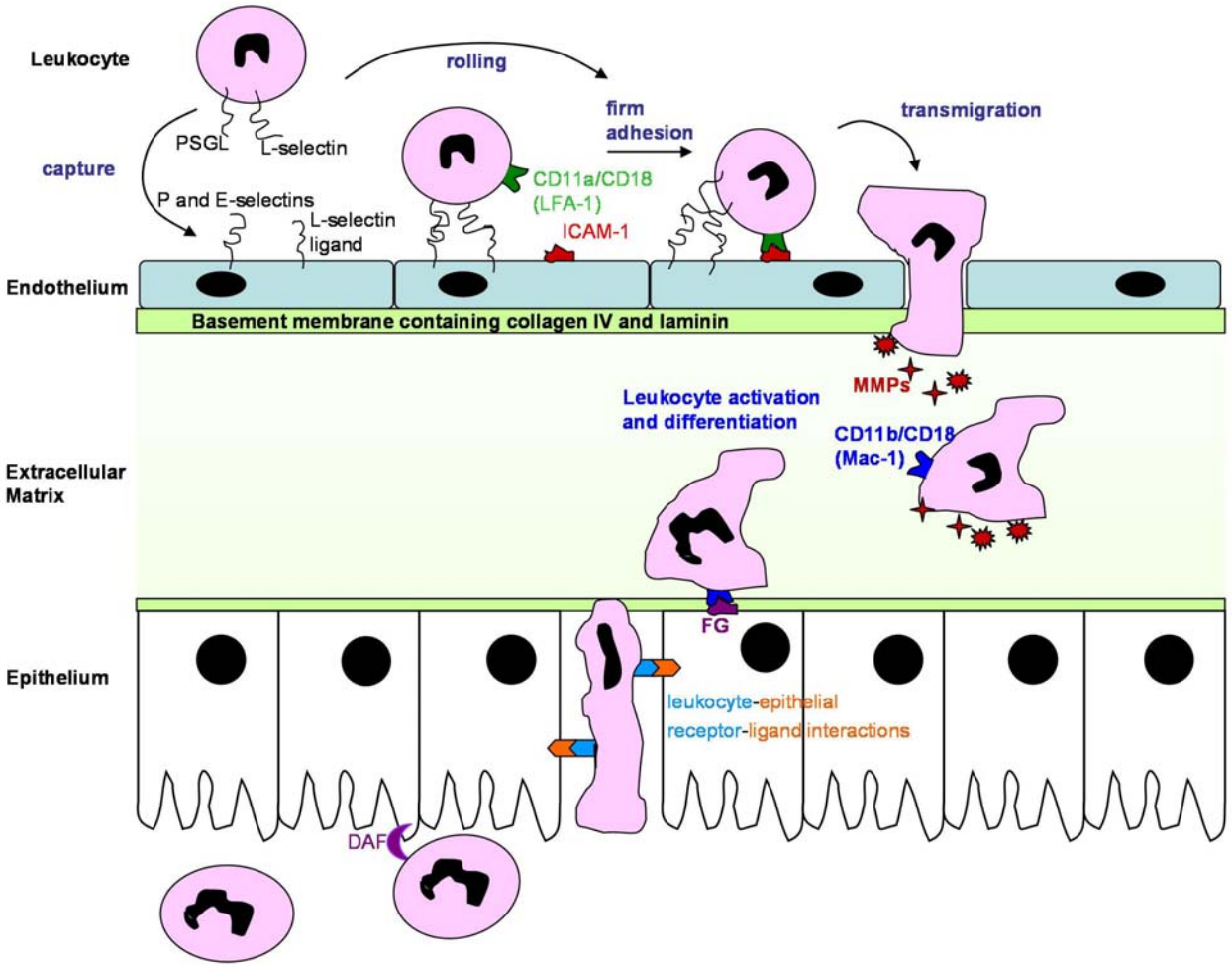

Figure 2. Leukocyte transmigration across endothelium and epithelium. While much is known about transendothelial migration, relatively little is understood about transepithelial migration. Upon inflammatory activation of the endothelium leukocytes are attracted to and captured on the endothelial surface. Capture and subsequent rolling over the endothelium is mediated by selectins and their ligands on either cell surface. During rolling, leukocytes become partially activated by chemokines/cytokines leading to firm adhesion that is mediated by the leukocyte integrin LFA-1 (CD11a/CD18) and endothelial ICAM-1. Firm adhesion initiates signaling cascades leading to the loosening of junctions and increased endothelial permeability. This enables leukocyte passage that includes several adhesive interactions within the paracellular space (see text). A detailed interactive description of diapedesis can be found online at http://bme.virginia.edu/ley/. Leukocytes migrate across extracellular matrices through the release of several matrix metalloproteases (MMPs). Leukocytes then adhere to the basolateral surface of the epithelium through binding interactions between leukocyte CD11b/CD18 and epithelial fucosylated glycoproteins (FG) and other yet to be defined receptors. Within the paracellular space leukocyte passage is facilitated by different adhesive interactions e. g. between leukocyte CD11b/CD18 or JAML and junctional proteins such as members of the JAM family or CAR. On the apical surface epithelial decay-accelerating factor (DAF or CD55) has been shown to play a role in neutrophil release from the epithelium.

structures comprised of vertical microvilli-like projections were observed that surround and guide leukocytes by redistributing leukocyte-integrins into an orientation parallel to the migratory direction (41). Disruption of this cup-like structure markedly decreased the rate of transmigration. However, for monocytes, the role of ICAMs has been shown to be restricted to the adhesion and movement on the apical endothelial surface (42).

Subsequent to firm adhesion of leukocytes to the endothelium, endothelial permeability needs to be altered as a prerequisite for transendothelial migration to occur. An increase in permeability can mainly be achieved by loosening of intercellular junctions to create gaps in the paracellular space. The ICAMs, VCAM and selectins on endothelial cells and β2-integrins on leukocytes have been shown to play a significant role in regulating endothelial permeability by transducing signaling pathways initiated through leukocyte adhesion. These signals lead to important intracellular changes including altered gene transcription and cytoskeletal rearrangment that ultimately cause increased permeability and trigger leukocyte transmigration (20,43). Increased vascular permeability has been shown to occur immediately after initial adhesion, and

independently of transmigration. For example, activated neutrophils added to human umbilical vein endothelial cells (HUVECs) in the absence of a transmigration-inducing stimulus has been shown to result in enhanced endothelial permeability which can be partly explained by tyrosine phosphorylation of β-catenin and VE-cadherin (44,45). A number of investigators have addressed the question of how the above permeability-increase is induced. In one study, dextran-sulfate was shown to inhibit neutrophil-induced increases in endothelial permeability and the results interpreted to suggest that cationic factors released from neutrophil granules bind to dextran-sulfate thereby sequestering them from permeability-inducing effects (46). The release of such permeability-inducing cationic molecules including cathepsin G, elastase, MMP-9 and azurocidin can be stimulated by crosslinking of neutrophil CD11b/CD18 or by binding of neutrophils to activated endothelium (47,48). Additional studies have shown that the neutrophil-derived molecule mainly responsible for increased permeability is azurocidin (48) which is a serin-protease homologue devoid of protease activity. The authors concluded that azurocidin functions in a paracrine fashion on the endothelial surface to alter permeability. The effects of azurocidin and other receptor-mediated signal pathways that are involved in regulating junctional integrity have been shown to be dependent on second messengers such as intracellular Calcium (Ca^{2+}, 49). In particular, adhesion of neutrophils to endothelia results in an increase in free cytosolic Ca^{2+} leading to increased vascular permeability and neutrophil transmigration (50-52). A major event that is mediated by this increase in free cytosolic Ca^{2+} after neutrophil adhesion is the activation of the Ca^{2+}-dependent myosin light chain kinase (MLCK) which in turn phosphorylates myosin light chain (MLC).

Phosphorylation of MLC induces a conformational change in the cytoskeleton, namely the contraction of the actin-myosin ring, which effectively retracts cells from one another leading to increased paracellular permeability (53-55). It has also been reported that actin-myosin ring contraction after MLCK phosphorylation is associated with increased neutrophil transendothelial migration (56). In this study, inhibition of MLCK phosphorylation reduced transmigration whereas phosphatase inhibition resulted in the accumulation of phosphorylated MLCK and significantly enhanced neutrophil transmigration. Neutrophil adhesion to endothelial ICAM has also been shown to activate the small GTP-binding protein Rho (57) which can activate Rho-associated kinase which in turn phosphorylates MLC to induce the same conformational changes as MLCK (58).

Another potent molecule in mediating outside-in signals following leukocyte adhesion is protein kinase C (PKC) which has also been shown to regulate endothelial permeability (59). However, direct activation of PKC does not lead to MLC phosphorylation but downregulation or inhibition of PKC decreases the level of MLC phosphorylation induced by thrombin suggesting that PKC can modulate endothelial contractility (60). Furthermore, activation of PKC-α has been linked to disassembly of VE-cadherin containing junctions and thus increased endothelial permeability (61). Accordingly, inhibition of PKC has been shown to reduce increases in pulmonary endothelial permeability induced by activated neutrophils (62). PKC also activates the tyrosine kinase $p60^{src}$ (63) that phosphorylates MLCK (64) and the AJC proteins β- and γ-catenin, p120 and SHP2 (65). Recently, cross-linking of and lymphocyte binding to VCAM-1 has been shown to activate PKC-α, whereas inhibition of PKC-α reduced transendothelial migration of spleen cells (66). PKC activation therefore appears to be an important event that precedes disassembly of the AJC and transmigration.

In addition to signaling mechanisms following adhesion of activated leukocytes to endothelial cells, the presence of pro-inflammatory cytokines released by activated leukocytes also contributes to increased endothelial permeability. In particular, IL-1β and TNF-α released by activated neutrophils have been shown to increase endothelial permeability, alter the molecular organization of tight junctions and lead to an increased expression of ICAM-1 at the apical surface that triggers further leukocyte recruitment (67,68). Additionally, it has been reported that activated monocytes, presumably through the release of TNF-α, IL-6 and IL-10, caused the formation of gaps between endothelial cells that led to enhanced monocyte transmigration (69). These studies indicate that both adhesion- and cytokine mediated signaling events facilitate leukocyte transmigration.

Even though the phosphorylation of MLC is an early critical signaling step that triggers the transmigration process, there is evidence that the apical junction complex (AJC), comprised of tight and adherens junctions (Figure 1), is altered immediately after leukocyte adhesion to endothelial apical ligands suggesting direct effects of adhesion on the junctions, presumably through different signaling pathways. In contrast to specialized endothelium e.g. the blood-brain barrier that forms extremely restrictive tight junctions, postcapillary venules, the main sites in most organs for leukocyte extravasation have comparably loose tight junctions. In these situations, evidence suggests that the adherens junction plays a key role in regulating leukocyte transmigration. All of the signaling mechanisms described thus far have been shown to result in cytoskeletal rearrangement allowing leukocytes to enter the paracellular space and transmigration to occur. A central step during transmigration through postcapillary venules is the reversible displacement of VE-cadherin that has been beautifully imaged in realtime using GFP-tagged VE-cadherin under defined flow conditions (70). This displacement of VE-cadherin occurred during both neutrophil and monocyte transmigration. Since VE-cadherin is the only transmembrane adhesion molecule within the paracellular space that does not facilitate but inhibit leukocyte transmigration (23,71), the displacement of VE-cadherin seems to be a crucial prerequisite for transmigration to occur. VE-cadherin at AJs associates with cytoplasmic catenins that are important mediators for the adhesive functions of cadherins in general. These cadherin/catenin complexes can be phosphorylated on tyrosine residues to alter cadherin-dependent adhesion. For example, stimulation of HUVECs in culture with vascular

endothelial growth factor (VEGF) induces tyrosine phosphorylation of VE-cadherin and associated catenins leading to increased endothelial permeability (72). Additionally, phosphorylation of certain residues in the cytoplasmic tail can block binding to catenins and thereby reduce VE-cadherin-mediated barrier function (73). By contrast, a protein tyrosine phosphatase (PTP) that specifically binds to VE-cadherin has been identified (VE-PTP) that can reverse VEGF-mediated VE-cadherin phosphorylation (74). In a recent study it was shown that antibody-mediated cross-linking of VCAM-1 activates PTP-1B and that inhibition of this activation inhibits VCAM-1-mediated lymphocyte transmigration (75). Since PTP-1B has been shown to regulate the N-cadherin/catenin complex (76) it appears that phosphorylation of the cadherin/catenin complex is an important mechanism regulating cadherin mediated cell adhesion and barrier function.

A remarkable difference between neutrophil and monocyte transendothelial migration is that neutrophils prefer to transmigrate at tri- or multicellular junctions within the endothelial cell layer. These junctions show small gaps in VE-cadherin staining (18,77). By contrast, monocytes seem to prefer bicellular borders where they induce reversible focal changes in the VE-cadherin complex (70,78). Another factor that affects the distribution of the VE-cadherin complex and thus vascular permeability are inflammatory cytokines such as TNF-α and IFN-γ that are secreted in the microvessels after an inflammatory response (79,80).

An important adhesion molecule of TJs is JAM-A, the first described of several members of the family of junctional adhesion molecules. JAM-A is expressed in both endothelial and epithelial cells at the TJ and has been reported to regulate paracellular permeability, cell polarity, cell adhesion, cell migration, angiogenesis, and leukocyte migration (81). Regarding junction disassembly, JAM-A has been shown to transiently redistribute from intercellular junctions after cytokine treatment which correlated with decreased leukocyte transmigration after combined treatment with TNF-α and IFN-γ (82). JAM-A has also been reported to engage leukocyte CD11a/CD18 by binding it to the membrane proximal domain of JAM-A and thereby triggering leukocyte transmigration (83) although these observations have not been confirmed by others. Another intriguing transient mechanism contributing to the passage of leukocytes was reported by Mamdouh and colleages (84). The authors described a new membrane compartment at the cell perimeter close to cell-cell borders that contains one third of the total cellular PECAM. When monocytes transmigrate, the authors reported that intracellular PECAM trafficking was altered in a way to direct it to the membrane at zones where monocytes transmigrate suggesting that PECAM engagement between monocytes and endothelial cells is required for monocyte transmigration. PECAM-1 is expressed on both leukocytes and endothelial cells. Furthermore, blocking PECAM-1 with mAbs on either cell type inhibits transmigration (85,86). Different Ig-domains of PECAM-1 seem to regulate different steps of diapedesis (87). When the first two domains are blocked, transmigration was shown to be reduced and monocytes accumulated on the apical surface, whereas blocking of domain six resulted in the accumulation of monocytes on the basolateral side where monocytes were arrested after diapedesis. Interestingly, PECAM-1 contains immunoreceptor tyrosine-based inhibitory motifs (ITIMS) (88). Upon receptor activation, ITIMs activate tyrosine phosphatases, such as SHP-1 or SHP-2 which dephosphorylate their substrates leading to inhibition of tyrosine kinase-mediated signaling events. PECAM-1 signaling has been shown to be more important in leukocytes during transmigration (89). In this respect, it has been shown recently that PECAM-1 may display a negative feedback function for LPS-induced activation of macrophages by inhibiting TLR-4 signaling (90) and that PECAM polymorphisms in monocytes influence monocyte adhesion to endothelial cells whereas polymorphisms in endothelial cells do not (91).

Other adhesion molecules have been shown to be involved in adhesion between leukocytes and endothelial cells during transmigration through the paracellular pathway. These include ESAM, CD99, JAM-B and JAM-C (2). ESAM is a JAM-related protein found in platelets and endothelial cells where it is engaged in homophilic interactions at the TJ and mediates cell aggregation (92,93). ESAM has been shown to regulate vascular permeability and neutrophil extravasation which may be regulated by the activation of the small GTPase Rho which is known to play a role in signaling events regulating transendothelial migration (94). CD99 is an endothelial adhesion molecule that is located at the lateral membrane but is not restricted to junctions. However, it has also been shown to play an important role during leukocyte extravasation since it has been reported that blockade of CD99 using a monoclonal antibody significantly reduced monocyte transendothelial migration without affecting monocyte adhesion (95). Additionally, JAM-B and JAM-C have also been reported to play roles during leukocyte extravasation by supporting different adhesive interactions between leukocytes and endothelial cells (96,97). JAM-C/JAM-B heterodimers seem important for the maintenance of endothelial barrier, since disruption of this interaction has been reported to make JAM-C accessible for leukocyte integrins and enhances leukocyte adhesion (96). Upregulation of JAM-C in cultured endothelial cells has recently been demonstrated to counteract adhesive properties of VE-cadherin whereas loss of JAM-C resulted in stabilized VE-cadherin-mediated interendothelial adhesion (97). In this study it has also been reported that such stabilization was dependent on the small GTPase Rap1 and the authors concluded that JAM-C may regulate vascular permeability by VE-cadherin-mediated adhesion. Recently, a role for JAM-B/JAM-C has been reported in monocyte reverse transmigration that may direct transmigrated cells back into the bloodstream (98). The authors showed that disruption of JAM-B/JAM-C interaction reduced the number of monocytes in inflammatory tissue while they detected an increased number of monocytes with a so-termed reverse-transmigratory phenotype in the blood. It was suggested that disruption of JAM-C-mediated monocyte retention

may be a novel mechanism responsible for reducing monocytes at sites of inflammation (98).

From the above overview of adhesion and signaling cascades it is evident that the migration of leukocytes across endothelial barriers requires a vast array of adhesion molecules that transduce multiple signaling pathways leading to loosening of junctions to enable diapedesis. The concerted action of a multitude of molecules in leukocytes as well as in endothelial cells is necessary to allow this complex process to occur. Even though much insight into this process has been gained from recent studies many of the molecular details remain unknown.

4. THE EPITHELIUM

4.1. Epithelial junctions

Mucosal surfaces of organs in the body are lined by single layers of highly specialized epithelial cells. The epithelial cells lining different organs are responsible for separating functionally distinct parts of the body from each other and the environment. Mucosal epithelia play critical roles in regulating exchange of water, solutes, nutrients and even cells. Similar to endothelial cells within blood vessels, epithelial cells are joined by tight and adherens junctions to regulate permeability. However, since epithelia protect the body from the outer environment, it is not surprising that epithelial junctions are generally tighter than those of non-specialized endothelia and comprised of a high number of intermolecular adhesion molecules. In contrast to endothelial cells, epithelial cells also form junctional structures called desmosomes that are comprised of specialized, cadherin-like adhesion molecules and are intracellularly connected to intermediate filaments (99). Desmosomes are localized below tight and adherens junctions in lower regions of the lateral membrane and thus represent the first intercellular barrier leukocytes encounter during transmigration since the polarity of transmigration is opposite of that which takes place across endothelia and occurs in a direction from the basolateral to the apical side of the epithelium. Desmosomes also play a central role in cellular signaling, regulating cell survival and barrier function (99-102).

Despite the tightness and high development of epithelial junctions, epithelial permeability also varies in different tissues (103). In tissues that are prone to invasion by pathogens from the exterior such as lung and colon there is constant immune surveillance taking place that reversibly alters epithelial permeability. This task is accomplished by tissue leukocytes that transmigrate from the basolateral to the luminal, apical epithelial surface where bacteria are collected and "evaluated" (104). For transepithelial migration to occur, there must be initial adhesion of leukocytes to basolaterally expressed epithelial adhesion molecules, and this occurs via the common leukocyte adhesion molecule CD11b/CD18 (Mac-1) (105). Following initial adhesion of leukocytes, subsequent adhesive events regulate the passage of leukocytes through the paracellular space by mechanisms that are still poorly understood. Since leukocyte-epithelial adhesion and transmigration through different specialized epithelia have already been described in detail elsewhere (10, 104, 106, 107), this review will focus on mechanisms different types of leukocytes exploit to achieve (reversible) opening of epithelial junctions in order to transmigrate. From the current literature, there are a number of ways that leukocytes can use to negotiate epithelial intercellular junctions: First, leukocytes may induce signaling pathways to trigger disassembly of junctions; second, leukocytes may release molecules that contribute to the reorganization or disruption of intercellular junction molecules; third, leukocytes might express surface molecules that compete for binding with junction-associated molecules and fourth, leukocytes may simply "force" their way through the paracellular space. There is experimental evidence that all of the above potential mechanisms may be involved.

4.2. Leukocyte epithelial interactions and their impact on junctional opening during transmigration

Leukocyte epithelial interactions and leukocyte transepithelial migration are much less well understood than leukocyte endothelial interactions and diapedesis. Since most studies in the literature are focused on one type of leukocyte, features that each different type of leukocyte exploits during adhesion to and migration across epithelial cell layers are more distinguishable. For this reason, we will discuss each leukocyte type separately in this section.

4.2.1. Neutrophils

In organs with high levels of microbial colonization such as the intestine, immune surveillance occurs that requires leukocyte transmigration (Figure 2). This process assures that pathogenic microorganisms cannot settle and negatively influence organ functions. This low level transmigration occurs even under non-pathologic conditions and utilizes mechanisms involving the reversible opening of epithelial junctions (108). However, under pathologic conditions, the process of immune surveillance is dysregulated and results in massive neutrophil transmigration that destroys epithelial junctions on a large scale, the consequence of which is epithelial injury and leakiness (10).

In contrast to migration across the vascular endothelium, leukocyte transepithelial migration is initiated by adherence to the basolateral side of the epithelium (Figure 1+2). Interestingly, adhesion of neutrophils to the basolateral surface of intestinal epithelium induces signal transduction events that appear superficially similar to those reported with neutrophil interactions with the apical surface of the endothelium. For example, in intestinal epithelium, neutrophil adhesion also leads to phosphorylation of MLCK (109). Similar to the events in endothelial cells, this phosphorylation appears to lead to the contraction of the actin-myosin ring, loosening of junctions and eventually to increased epithelial permeability (109).

An important mechanism by which neutrophils influence epithelial permeability is the release of proteases which, upon degranulation, can be directed onto the epithelial cell surface to cleave and disengage adhesion molecules such as E-cadherin (110). Additionally,

neutrophil-derived proteases activate protease-activated receptors (PARs) which are known to play an important role in cellular signal transduction (111). Activation of PARs has also been shown to lead to the activation of MLCK, thereby affecting the molecular organization of epithelial junctions and increasing epithelial permeability (110,112).

Another process that might play a role during the reversible opening of epithelial junctions is the internalization of junctional proteins that occurs after long-term exposure to inflammatory cytokines such as IFN-γ (113), which is prominent in inflammatory diseases and is abundantly secreted by leukocytes. Internalization of AJC proteins can occur via endocytosis (114). However, internalization after IFN-γ treatment seems to be mediated more specifically by macropinocytosis which is a particular type of endocytosis (113). After internalization, junctional proteins can either be delivered into recycling endosomes and retargeted to the AJC or they are targeted to late endosomes and degraded leading to severely compromised junctions (102). Thus, depending on the intracellular fate of internalized AJC proteins, junctions can be resealed or completely disassembled leading to epithelial leakiness.

Similar to events occurring during diapedesis, adhesion molecules play a central role during transepithelial migration of leukocytes. However, in contrast to interactions with the endothelium, initial adhesion of neutrophils to the epithelial cell surface is selectin-independent, and mediated by neutrophil CD11b/CD18 but not CD11a or CD11c (115). Naturally, this adhesive interaction is with the basolateral epithelial surface, and epithelial counterreceptors are still not characterized. As evident from vascular endothelial studies, one attractive candidate is ICAM-1. However, this CDllb-ligand has been shown to be expressed mainly on the apical side of intestinal epithelia and only under inflammatory conditions (105) thus its location in the wrong place renders it an unlikely candidate for transmigrating neutrophils in the intestines. Interestingly, others have shown that under certain inflammatory conditions ICAM-1 may be expressed in a physiologically relevant fashion for transmigrating neutrophils on the basolateral surface of kidney epithelium (116). Furthermore, it has also been reported that activation of airway epithelium by adherent neutrophils increases epithelial permeability and involves ICAM-1 (117). The authors also found that blocking of ICAM-1 using monoclonal antibodies reduced epithelial transmigration. Thus, ICAM-1 may serve as a physiologically relevant ligand during neutrophil interactions with epithelial cells in certain organs. Other studies have suggested that there are additional epithelial ligands for neutrophil CD11b/CD18 including as of yet uncharacterized fucosylated glycoproteins that may mediate early binding events during transepithelial migration (118,119). Since these early adhesive events are poorly understood, the first signaling events that lead to the initial loosening of epithelial junctions are also unclear.

Following firm adhesion on the basolateral surface neutrophils extend pseudopods into the paracellular epithelial space. Here, neutrophils first encounter desmosomes that do not exist in endothelia. However, the role of desmosomes during neutrophil transmigration is still unclear. Binding interactions between CD11b/CD18 and a JAM family member reported to localize to epithelial desmosomes termed JAM-C have been suggested to play a role in regulating leukocyte transepithelial migration (120).

After having passed desmosomes, neutrophils encounter adherens and tight junctions where similar binding interactions occur as already discussed for the endothelium. Another receptor ligand pair consists of the epithelial tight junction protein coxsackie- and adenovirus receptor (CAR) and leukocyte JAML (JAM-like protein) which have also been described to facilitate neutrophil transepithelial migration (121). Additionally, in a recent study, JAM-A was identified *in vivo* as a key molecule regulating epithelial permeability and mucosal inflammation (122). Current evidence suggests that JAM-A is not only involved in adhesive interactions but also transduces signaling events to regulate epithelial permeability. In particular, JAM-A has been reported to play roles in regulation of cell shape and β1-integrin expression through activation of the small GTPase Rap1 (123), which has been shown to enhance endothelial barrier function and inhibit transmigration (124). It is thus tempting to speculate that JAM-A plays an important role in signaling events that facilitate leukocyte passage across epithelia by transient alterations in paracellular permeability.

Another signaling receptor-ligand pair that has been shown to play a role in regulating the rate of neutrophil transepithelial migration involves CD47 (125). Similar to CD99 in endothelial cells, CD47 is a transmembrane adhesion molecule that is not restricted to a certain junctional region. Epithelial CD47 has been shown to engage leukocyte signal recognition protein (SIRP)-α and facilitate neutrophil transepithelial migration through poorly characterized signaling events (126,127).

In conclusion, more and more evidence is accruing to indicate that neutrophil transepithelial migration is a complex process requiring multiple adhesive steps in which numerous different adhesion molecules are involved. These adhesive interactions not only transduce signal pathways that lead to the loosening of junctions but also ensure the maintenance of barrier integrity under physiologic conditions by tightly interposition the neutrophils within the paracellular space of the epithelium.

4.2.2 Macrophages

Macrophages derived from human blood monocytes perform many tasks related to tissue injury and repair. Within tissues, monocytes differentiate into resident macrophages such as Kupffer cells, alveolar macrophages or osteoclasts that do not significantly affect epithelial barrier function. However, during inflammatory responses freshly recruited blood monocytes not only differentiate but are also classically activated, meaning they secrete a pro-inflammatory cytokine and chemokine cocktail which greatly affects the epithelium (128). For example, freshly recruited macrophages activated by LPS and cocultured

with the intestinal epithelial cell line T84 have been shown to cause a drop in transepithelial electrical resistance (TER) (129). Additionally, macrophages isolated from the lamina propria (LP) of individuals with active Crohn's disease altered epithelial secretory and barrier parameters, whereas LP-macrophages of healthy persons did not affect epithelial function (130). However, active transmigration of macrophages across healthy intestinal epithelium has not yet been described. Nevertheless, macrophages migrate within the LP and also through the basement membrane if the epithelium is removed from colon tissue (131). The authors concluded that transmigration of macrophages in the intestines can only occur if the epithelium is either injured or dysfunctional, both of which stages occur during acute phases of IBD. However, this would exclude that macrophages are able to actively open up epithelial junctions in the intestines. The situation is different in the lung. In mice, macrophages are capable of migrating along and across airway surfaces in order to perform immune surveillance and to regulate the immune response (132). Thus, while this process has been poorly characterized, transepithelial migration of macrophages in the lung is clearly an important step in pulmonary immune function.

In conclusion, the interactions of macrophages with epithelial cells are barely investigated. However, it is evident that macrophages in the intestines mainly reside at the basolateral site of the epithelium, whereas active transmigration has been observed in the lung. Generally, macrophages influence the permeability of the epithelial barrier and the recruitment of transmigrating neutrophils by the release of chemotactic factors and proinflammatory cytokines.

4.2.3 Dendritic cells

Another important innate immune cell capable of reversibly opening epithelial junctions is the dendritic cell (DC). DCs are specialized antigen-presenting cells that can initiate the immune response against invading pathogens (36). DCs do not necessarily have to transmigrate to accomplish their tasks. It was demonstrated in two independent studies that this cell type can penetrate the gut epithelium to sample bacteria from the gut lumen by sending out dendrites (133,134). This process might be facilitated by the expression of tight junction proteins on the dendrite surface that may enable homophilic binding interactions with epithelial junctions and may ensure the sealing of the paracellular space and maintenance of barrier function (133). Additionally, it was reported that dendritic cells express E-cadherin and β-catenin. Homophilic E-cadherin interactions may thus serve as handholds for DCs prior to the opening of tight junctions. This hypothesis is substantiated by earlier findings that DCs could not penetrate tight junctions when cells were applied to the apical surface (135). Similar observations of dendritic cell-epithelial penetrations were recently made in the lung where DCs were shown to penetrate lung epithelia to directly collect putative pathogens for "evaluation" (136). In doing so, DCs may collaboratively interact with alveolar macrophages to form a transepithelial cellular network against invading pathogens (137). In addition, certain populations of airway dendritic cells have also been shown to express tight junction proteins for direct homophilic adhesive interactions in order to directly access the airway and to simultaneously sustain barrier function (138). DCs may also exploit matrix metallo-proteinases such as MMP-9 to migrate through epithelial junctions. Indeed, it has been reported that MMP-9-deficient DCs have impaired transepithelial migration which was, in part, explained by an altered turnover pattern of the tight junction protein claudin-1 (139). These studies have opened a new intriguing area in the field of leukocyte-epithelial interactions but still await rigorous confirmation of these processes in different organs.

5. CONCLUSION

Leukocytes play a major role in host defense and must migrate through endothelial and epithelial junctions to accomplish many of their tasks. This process of transjunctional migration is crucial to host survival but also results in significant dysfunction in many diseases. Thus, disruption of junctions by leukocytes must be highly regulated. It is evident that adhesion of leukocytes to the apical side of endothelial cells or the basolateral side of epithelial cells, respectively, results in signaling events that lead to cytoskeletal rearrangement and to the opening of intercellular junctions. Under regulated immune responses, subsequent adhesive steps within the paracellular pathway allow the passage of leukocytes with a parallel "sealing" of the barrier that guarantees stable barrier function. This involves the binding of junctional molecules to receptors on the leukocyte surface. However, many inflammatory conditions are characterized by excessive leukocyte transmigration leading to the disruption of junctions and the massive loss of barrier function. A number of the underlying signaling events and receptor-ligand pairs crucial to regulating this response have been identified and have contributed to the understanding of the process of transmigration. However, our understanding is far from complete. Many questions need better answers to fully comprehend these complex processes. Epithelial transmigration is much less well understood than migration across vascular endothelium and should be emphasized given its importance during the immune response. Further characterization of the regulation of junctional disassembly and transmigration may allow the development of new treatment strategies of inflammatory diseases with robust leukocyte transmigration and severely compromised barrier function.

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