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JAM related proteins in mucosal homeostasis and inflammation

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Abstract

Mucosal surfaces are lined by epithelial cells that form a physical barrier protecting the body against external noxious substances and pathogens. At a molecular level, the mucosal barrier is regulated by tight junctions (TJs) that seal the paracellular space between adjacent epithelial cells. Transmembrane proteins within TJs include Junctional Adhesion Molecules (JAMs) that belong to the CTX (Cortical Thymocyte marker for *Xenopus*) family of proteins. JAM family encompasses three classical members (JAM-A, -B and -C) and related molecules including JAM4, JAM-Like protein (JAM-L), Coxsackie and Adenovirus Receptor (CAR), CAR-Like Membrane Protein (CLMP) and Endothelial cell-Selective Adhesion Molecule (ESAM). JAMs have multiple functions that include regulation of endothelial and epithelial paracellular permeability, leukocyte recruitment during inflammation, angiogenesis, cell migration and proliferation. In this review, we summarize the current knowledge regarding the roles of the JAM family members in the regulation of mucosal homeostasis and leukocyte trafficking with a particular emphasis on barrier function and its perturbation during pathological inflammation.

Keywords

JAMs; Tight junction; Leukocyte trafficking; Inflammatory Bowel Disease

Introduction

Junctional Adhesion Molecules (JAMs) are glycoproteins that belong to the immunoglobulin superfamily (IgSF) composed with two extracellular immunoglobulin-like domains (V-C2 type Ig-domains) [1], one transmembrane spanning segment and a cytoplasmic tail of variable length. The JAM family is composed of three closely related proteins (JAM-A [2,3], JAM-B [4,5] and JAM-C [6,7]) which display up to 35% of identity at the amino acid level and share a short intracellular domain of variable length (40–50 residues) containing a class II PDZ-binding-motif at the C-terminus [8]. The JAM family also encompasses a subgroup of related proteins consisting of JAM4 [9], JAM-Like (JAM-L) protein [10], Coxsackie and Adenovirus Receptor (CAR) [11], CAR-Like Membrane Protein (CLMP) [12] and Endothelial cell-Selective Adhesion Molecule (ESAM) [13]. They differ from the classical JAMs by the presence of a long cytoplasmic tail (98–120 residues) ending with a class I PDZ-binding motif, except for JAM-L and CLMP [8,12]. Since Class I

and II PDZ-binding motifs are involved in interactions with scaffold proteins that are linked to the actin cytoskeleton and signaling pathways, JAMs and related proteins are positioned to associate with different cytoplasmic proteins responsible for distinct functions. As summarized in table 1, JAM family members display a broad tissue distribution and are expressed in various cell types. Overall, JAMs are generally expressed in subsets of leukocytes in addition to endothelial and epithelial cells. However, some show more restricted expression such as JAM-L which is expressed exclusively on subsets of leukocytes, and CLMP which is expressed only on subsets of epithelial cells. Because of their presence in endothelia and leukocytes, JAM family members have stimulated intensive investigations related to their role in vascular homeostasis and leukocyte trafficking. To date, JAMs are well-accepted as important molecules controlling vascular permeability, leukocyte transendothelial migration, angiogenesis and tumor progression [14–16]. While expressed on epithelia, less is known about JAM function in mucosal homeostasis. This review provides an overview of the current knowledge on the role of JAMs and related proteins in the maintenance of the mucosal epithelial barrier and the recruitment of leukocytes through the epithelium during inflammation with particular emphasis on the intestinal tract.

The role of JAM family members in mucosal epithelial barrier function

The intestine is a good example of an organ with crucial mucosal function. It is lined by a single layer of epithelial cells that forms a selective physical barrier allowing the passage of nutrients and solutes while protecting the body against external antigens. Underlying the epithelium is the lamina propria composed of capillaries, immune cells and mucosa-associated lymphoid tissue (MALT) which play a central role in gut immune responses (Figure 1a). Epithelial cell-cell contacts consist of specific junctional complexes that include tight junctions (TJs), adherens junctions (AJs) and desmosomes (Figure 1b). TJs form a seal at the apical-most region of the epithelial lateral membrane [17]. Three types of transmembrane proteins constitute the TJ protein complex: JAMs and the tetraspan proteins, occludin [18] and claudins [19]. All of these transmembrane proteins are linked to cytoplasmic scaffolds [8] and signaling proteins [20,21]. Claudins are barrier-forming components of the TJ and play a central role in the physical regulation of paracellular permeability [22] while the role of occludin in regulation of barrier function is controversial. There is evidence to suggest that occludin functions as a signaling protein to regulate epithelial differentiation and TJ barrier stability [23–26]. Other studies have provided strong evidence that JAM family members regulate TJ assembly and barrier function in epithelia.

JAM-A regulation of epithelial TJ formation and barrier

Among the classical JAM family members, current evidence indicates that only JAM-A is expressed on mucosal epithelial cells and is directly involved in TJ formation and maintenance. The expression of JAM-C on mucosal epithelia is less clear since multiple anti-JAM-C antibodies have been found to cross-react with a phosphorylated form of cytokeratin-8 and thus label desmosomes. An anti-JAM-C monoclonal antibody validated as highly specific did not detect JAM-C in epithelial cells from several normal human tissues including colon and lung, suggesting that JAM-C may not be abundantly expressed in

mucosal epithelia [27]. By contrast, analysis of the subcellular localization of JAM-A in human-colonic mucosa demonstrates prominent staining of epithelial TJs [28]. Similarly, electron microscopy and immunofluorescence staining of cryosections of mouse duodenum demonstrated robust JAM-A expression in epithelial cells [3]. Epithelial TJ expression was confirmed *in vitro*, in freeze-fracture replicas of MDCK cells, in which endogenous JAM-A molecules were found tightly associated with TJ strands [29]. Interestingly, JAM-A failed to reconstitute TJ strand-like structures when overexpressed in mouse fibroblasts lacking TJs while it has been shown for claudin-1 in the same cellular system. This result suggests that unlike claudin-1, JAM-A is not directly involved in the formation of TJ strands and has a different role [29,30]. Indeed, JAM-A depletion by small interfering RNA increased barrier paracellular permeability as assessed by a decrease in the transepithelial resistance (TER) in human intestinal epithelial cells from various origins [31,32], suggesting that JAM-A plays a role in regulation of barrier. This observation has been validated by *in vivo* studies where, JAM-A- deficient mice demonstrated increased intestinal epithelial paracellular permeability as determined by the diffusion of 4kDa-FITC-dextran and decrease of TER compared to wild-type mice [31]. Furthermore the treatment of normal mice with JAM-A function-blocking antibodies has been reported to increase the intestinal permeability to Evans blue and reduce the TER [32]. In addition JAM-A-deficient mice are more susceptible to Dextran Sulfate Sodium (DSS)-induced colitis compared to control mice, supporting the hypothesis that JAM-A depletion facilitates the development of colitis in part by inducing a leaky mucosal barrier [31,32].

The molecular mechanisms by which JAM-A controls epithelial barrier is beginning to be understood from structural data and identification of molecular partners within the TJ complex. Based on crystallographic structural studies, JAM-A is reported to form *cis*-homodimers (on the same cell surface) through ionic interactions within a conserved dimerization motif, R(V,I,L)E in the distal Ig-like extracellular domain [33,34]. In the murine crystal structure, JAM-A *cis*-homodimers have been shown to engage in trans-interactions with JAM-A dimers on the opposite cell surface, however the putative site of trans-dimerization has not been defined [33]. The importance of JAM-A *cis*-homodimers in regulation of epithelial barrier function has been shown through the use of JAM-A function-blocking antibodies that inhibit the formation of JAM-A *cis*-homodimers and prevent the recovery of transepithelial resistance (TER) after transient calcium depletion of intestinal epithelial cells [28,35]. JAM-A function-blocking antibodies have also been shown to increase paracellular permeability to 10kDa-FITC-Dextran after transient calcium depletion in retinal pigment epithelial cells [36], suggesting a role of JAM-A *cis*-homodimers in the regulation of TJ function in various types of epithelial cells. Moreover, JAM-A mutant proteins that prevent *cis*-dimerization (deletion of the distal Ig-loop or point mutation E61R/K63E) fail to accumulate at intercellular contacts resulting in diffuse localization over the entire plasma membrane of CHO cells, strongly suggesting that the formation of *cis*-homodimer stabilizes JAM-A at intercellular junctions [35].

JAM-A mutants lacking the extracellular domain or the membrane distal V-type Ig domain have also been reported to impair the ability of MDCK-II cells to form spherical structures (cysts) in 3D collagen gels, which correlated with the establishment of the apico-basal

polarity [37]. Similar results were obtained with knockdown of JAM-A by siRNA approaches, suggesting that JAM-A is involved in the formation of epithelial cell polarity. A physical interaction between JAM-A and Par3, a component of the polarity complex (Par3/Par6/aPKC) is strong evidence for its role in epithelial cell polarity. Indeed, JAM-A has been shown to bind to Par3 through the C-terminal PDZ binding motif resulting in the recruitment of the polarity complex Par3/Par6/aPKC to the TJ [29,38,39]. Interestingly, aPKC has also been reported to induce the phosphorylation of JAM-A at serine 285 which stabilizes JAM-A at the TJ and is indispensable for the development of a functional barrier [40]. These findings strongly suggest that the C-terminal PDZ motif of JAM-A is crucial for both the recruitment of JAM-A at TJs and in mediating its function. In line with this observation, JAM-A can also interact through its C-terminal PDZ-binding motif with several TJ-scaffold proteins such as Zonula Occludens proteins (ZO) [41–43], Afadin [44,45], MUPP1 [46] and CASK [47]. These molecular interactions may form a large protein complex that stabilize JAM-A at the TJ and participate in JAM-A-dependent TJ regulation. Recently, we described a molecular pathway involving JAM-A and the scaffold molecule afadin that regulates epithelial barrier function. JAM-A was shown to associate with ZO-2, afadin and PDZ-GEF1 to control activation of the small GTPase Rap2c which, in turn, regulates epithelial permeability to large molecular weight solutes by modulating levels of RhoA and contraction of the apical actomyosin cytoskeleton [42]. Consistent with this model, JAM-A-deficient mice and JAM-A deficient human epithelial cells show enhanced paracellular permeability to large molecules (40kDa), indicating that JAM-A plays a role in regulation of both intestinal epithelial permeability to small and high-molecular weight solutes. Furthermore, these observations are in keeping with the involvement of the RhoA signaling pathway playing a central role in regulation of contraction of the perijunctional actomyosin network thereby expanding the paracellular space between epithelial cells [48]. In further support of this model, mice that have epithelial-targeted loss of afadin have enhanced RhoA activity [49] and phenocopy JAM-A knockout mice by demonstrating increased paracellular permeability across the intestinal epithelium and enhanced susceptibility to DSS-induced tissue injury [31,50]. Moreover the absence of afadin does not alter the localization of JAM-A and TJ proteins (occludin, ZO proteins) strongly suggesting that afadin is a downstream effector of JAM-A. Altogether, these studies highlight a crucial role of the JAM-A/afadin/Rap2 and RhoA signaling in controlling intestinal epithelial paracellular permeability to large molecules.

Loss of JAM-A has also been reported to upregulate the expression of claudin-10 and -15 suggesting a role of JAM-A in regulating the molecular composition of TJs and hence permeability to small solutes [31,32]. It is now appreciated that specific combinations of claudins can pair to form a tight or leaky barrier to ions and small molecules. For example, claudin-2 and -10 tend to make TJs leakier while claudin-4, -5, -7 and 15 tend to strengthen the barrier [51]. However, the signaling pathway(s) by which JAM-A regulates claudin-10 and -15 expression is not understood. It is possible that transcription factors such as hepatocyte nuclear factor-4 α (HNF-4 α), which is known to regulate the expression of claudin-15 and participate in the establishment of epithelial cell polarity is involved in JAM-A function [52,53]. Since HNF-4 α has been reported to induce JAM-A gene expression [54], we speculate that in absence of JAM-A, a compensatory mechanism involving HNF4 α

could stabilize the epithelial barrier by regulating expression of certain claudins. Consequently, a feed-back loop might involve JAM-A and HNF-4 α to maintain epithelial barrier homeostasis. More studies are clearly needed to examine these possibilities.

JAM-related proteins in epithelial TJ formation and maintenance

JAM4 is expressed in intestinal epithelial cells and distributed at the apical membrane as well as at TJs in sections of rat small intestine as demonstrated by immunohistochemistry and electron microscopy [9,55]. Similar to JAM-A, the localization of JAM4 at TJs is dependent on the distal extracellular Ig-like domain and JAM4 can form cis-homodimers [55]. Interestingly, the C-terminal cytosolic PDZ-binding motif of JAM4 does not bind to ZO-1 but to MAGI-1 (membrane-associated guanylate kinase protein), a scaffold protein associated with TJs that is also expressed in epithelial intestinal cells [9]. JAM4 has been reported to recruit MAGI-1 to TJs in mouse fibroblasts and in MDCK cells [9,55], that in turn recruits ZO-1 and occludin to TJs suggesting a role for JAM4 in TJ assembly and stabilization. Moreover, overexpression of JAM4 in mouse fibroblasts was found to induce cell aggregation [9,55] and JAM4 overexpression in CHO cells can reduce the paracellular diffusion of 40kDa-FITC-dextran [9]. Altogether, these findings suggest that JAM4 engages in homophilic interactions and is positioned to play a role in the regulation of epithelial barrier function. Further investigations are needed to clarify the mechanisms by which JAM4 controls the formation and maintenance of the mucosal epithelial barrier.

CAR (Coxsackie and Adenovirus Receptor) was first described as a receptor for type B coxsackievirus and subgroup C adenovirus and has been extensively studied for adenovirus-based therapy [56,57]. CAR has been shown to localize and physically interact with ZO-1 and Multi-PDZ Domain Protein-1 (MUPP-1). CAR is described to recruit the latter within TJs of intestinal epithelial cells, indicating that CAR is a component of the apical junctional complex [58–60]. Interestingly, unlike JAM-A, -B and -C, CAR does not bind to PAR-3 [61]. Overexpression of CAR molecules in CHO cells increased cell aggregation, and two crystal structures have confirmed that CAR can engage in homotypic interactions [62,63]. Addition of soluble CAR proteins to MDCK cell monolayers inhibited the recovery of TER after calcium depletion, suggesting a role for CAR in regulation of epithelial TJs barrier assembly [58]. Recently, two studies have described a mechanism by which CAR may regulate epithelial intercellular junctions. CAR was found to control E-cadherin stability at cell-cell contacts by promoting the internalization of E-cadherin [64,65]. CAR phosphorylation by PKC δ was shown to regulate Src-dependent endocytosis of E-cadherin at cell junctions. Specifically, CAR-dependent E-cadherin endocytosis occurred when CAR is not phosphorylated but PKC δ -dependent phosphorylation resulted in the recovery of E-cadherin at adherens junctions (AJs). These data suggest that PKC δ mediated phosphorylation of CAR maintains E-cadherin at junctions and may serve to stabilize epithelial cell-cell adhesion [65]. Furthermore, the above observations suggest that CAR may act preferentially on the maintenance of epithelial AJs instead of TJs. Interestingly, conditional knockout of CAR in mice resulted in the dilatation of the intestine without change in the overall length of the gastrointestinal tract or signs of colitis. Moreover, the integrity of the intestinal epithelium in CAR deficient mice is maintained as no defect in epithelial paracellular permeability to 4kDa-FITC-Dextran or localization of occludin in

epithelial TJs was observed. These findings suggest that loss of CAR does not affect formation of the TJ protein complex or maintenance of the epithelial barrier [66]. It is tempting to speculate that the loss of CAR in the intestine may be compensated by expression of other JAM family members or CAR-Like Membrane Protein (CLMP) which is closely related to CAR.

CLMP was identified 10 years ago as a new member of the IgSF family and has 31% of protein identity with CAR, its closest homologue [12]. CLMP transcripts have been abundantly detected in the small intestine, and the protein is expressed mainly in epithelial cells [12]. Importantly, loss of function mutations in CLMP have been found in patients with Congenital Short Bowel syndrome, a disease characterized by a shortening of the small intestine and impaired intestinal absorptive function [67], suggesting a role for CLMP in the embryonic development. Knockdown of CLMP *in vivo* in zebrafish also resulted in shortening of the intestine and the absence of goblet cells, confirming that CLMP is necessary for small intestinal development [67]. However, the function of CLMP in adult intestine is largely unknown. To date, it has been reported that endogenous or overexpressed CLMP proteins colocalizes with ZO-1 and occludin in Caco-2 and MDCK cells respectively, suggesting that CLMP is a component of the epithelial TJ complex [12]. Moreover, CLMP overexpression mediated cell aggregation in CHO and enhanced TER of MDCK monolayers [12]. These observations strongly suggest that CLMP may be involved in regulating cell-cell contacts through homophilic interactions thereby participating in controlling epithelial TJ barrier function. Despite these interesting observations, much more work is needed to better understand the role of CLMP in mucosal barrier function.

JAM family members in leukocyte recruitment and mucosal inflammation

JAMs in leukocyte transendothelial migration

The gastrointestinal tract is continuously in contact with exogenous antigens (pathogens, dietary substances) and commensal microorganisms. Pro-inflammatory signals can arise from epithelial cells, intraepithelial lymphocytes and dendritic cells triggering the recruitment of leukocytes from the bloodstream toward the mucosal surface [68,69]. During recruitment, leukocytes migrate out of mucosal microcirculation by a process termed transendothelial migration (TEM) which is a multistep process involving specific adhesive events between the cytokine-activated endothelium and leukocyte surface proteins (See for review [70]). As summarized in table 2, the TEM process involves numerous families of molecules such as selectins, integrins and Cell Adhesion Molecules (CAMs) that engage in heterophilic interactions between endothelial cells and leukocytes (except PECAM-1 involved in homotypic binding). JAM family members have been reported to engage in both, homophilic as well as heterophilic adhesive interactions and their involvement in the leukocyte TEM process has been described in numerous studies by *in vitro* and *in vivo* approaches (as discussed below). Endothelial JAM members (JAM-A, -B, -C, ESAM and CAR) are found concentrated at the apical intercellular junction with TJ components [61,71–75]. It has been reported that inflammatory cytokines (tumour necrosis factor (TNF)- α and interferon (IFN)- γ) induce redistribution of JAM-A from the TJ toward the luminal surface of the endothelium [76]. This observation suggests that JAM-A can be accessible to mediate

leukocyte adhesion on endothelial cells through homophilic interactions or heterophilic binding to integrin LFA-1 (CD11a/CD18) on leukocytes [77–79]. JAM-A, has been shown to be involved in leukocyte TEM in various murine models of inflammation, such as air pouch skin inflammation [3], cytokine-induced meningitis [80], hepatic and heart ischemia-reperfusion (I/R) injury [81,82] and in response to interleukin-1 and I/R injury in cremasteric venules [83]. JAM-A has been reported to regulate leukocyte TEM in a stimulus-dependent manner [83]. In addition, Woodfin *et al* has described sequential events for leukocyte TEM involving ICAM-2, JAM-A and PECAM-1. In this model, ICAM-2 is responsible for leukocyte engagement at intercellular junction while JAM-A facilitates leukocyte migration through the paracellular route and PECAM-1 controls the migration across the vascular basement membrane [83,84]. The specific role of JAM-A on leukocytes has been investigated using polymorphonuclear neutrophils (PMNs) lacking JAM-A that showed strong adhesion to the endothelium and defective polarization as well as directional movement toward a chemotactic stimulus [81,85]. Furthermore, dendritic cells lacking JAM-A have been described to migrate more efficiently into lymph node tissue associated with enhanced activation of adaptive immunity [86] suggesting that JAM-A regulates cell motility in a cell type dependent manner. Overall, JAM-A seems to play distinct roles in the endothelium compared to leukocytes in facilitating TEM in response to specific inflammatory stimuli. However, the relative contribution of endothelial JAM-A and leukocyte JAM-A in the TEM process is not fully understood.

Unlike JAM-A, JAM-B is not expressed on leukocytes and engages in heterophilic interactions with two ligands on leukocytes, JAM-C [6] [87] and the integrin VLA-4 (CD49d/CD29) [88]. While JAM-B and JAM-C contain a conserved dimerization motif as seen in JAM-A and may form cis-homodimers within endothelial intercellular contacts, there is evidence to suggest that the affinity of heterophilic JAM-B/JAM-C interactions is greater than the homophilic interaction JAM-C/JAM-C [89]. In a model of cutaneous inflammation, neutralizing antibodies against either JAM-B or JAM-C decreased leukocyte infiltration, and the combined antibody treatment had synergistic effect indicating that JAM-B and -C may have distinct functions in leukocyte TEM [90]. In line with a distinct role of JAM-B, antibody blocking experiments in mice showed that JAM-B is involved in regulation of rolling and firm adhesion of T lymphocytes under shear stress, and this function is dependent on VLA-4 integrin but not JAM-C [91]. Endothelial JAM-C has been reported to bind to the integrin Mac-1 (CD11b/CD18) expressed on leukocytes [92] and to play a role in leukocyte TEM in both *in vitro* systems and *in vivo* models of inflammation such as peritonitis, acute pulmonary inflammation, acute pancreatitis and ischemia reperfusion injury [93–98]. Recent evidence suggests that JAM-C expression promotes leukocyte TEM by preventing reverse transmigration of leukocyte in an abluminal to a luminal direction by controlling the polarization of cell migration [99,100]. Moreover, JAM-C has been reported to regulate vascular permeability as revealed in JAM-C knockdown endothelial cells by the strengthening of VE-cadherin-mediated cell adhesion (in a manner dependent on the GTPase Rap1) and reduction of vascular permeability. Conversely, factors that induce vascular permeability such as vascular endothelial growth factor (VEGF) have shown an accumulation of JAM-C into cell-cell borders, MLC phosphorylation, actomyosin contraction and increased paracellular permeability [101]. JAM-C therefore appears to be an

important regulator of leukocyte TEM by modulating both, leukocyte-endothelial interactions and vascular permeability.

Among JAM related proteins expressed on the endothelium (ESAM and CAR), ESAM has been shown to play a role in regulating neutrophil TEM independent of the type of inflammatory stimulus [102,103]. ESAM has also been shown to regulate macrophage infiltration in a murine model of peritonitis and atherosclerosis [104]. *In vitro* adhesion assays showed that THP-1 monocytic cells, which did not express ESAM, bound to the immobilized ESAM and TEM was reduced when endothelial ESAM was depleted. This observation suggests that ESAM may interact with heterophilic ligand(s) on monocytes distinct to VLA-4, MAC-1, LFA-1 or JAM-A [104]. Interestingly, knockdown of ESAM expression in endothelial cells *in vitro*, resulted in reduced levels of activated Rho. The latter when activated can lead to enhanced permeability by destabilization of endothelial TJs [103]. This finding suggests the presence of ESAM by activation of Rho may destabilize the intercellular contacts. Furthermore, the authors found that the vascular permeability in ESAM null mice is less affected compared to wild-type mice and after treatment with agents that increase vascular permeability. Thus, the resultant changes in permeability were less in the absence of ESAM. Consequently, these results suggest that ESAM (similar to JAM-C) may promote leukocyte TEM by the transducing signals to open endothelial cell contacts [103]. Conversely, the depletion of ESAM has been also reported to increase endothelial permeability *in vitro* in response to high glucose and in ESAM-null mice in a model of diabetic nephropathy [105]. Overall these findings strongly suggest that ESAM plays an important role in regulation of endothelial barrier permeability. Further investigations are needed to understand in which context the loss of ESAM may induce an increase or decrease in vascular permeability. Finally, CAR has been shown to play a role in leukocyte-endothelial cell interactions through heterophilic binding to JAM-L expressed on leukocytes, namely neutrophils, monocytes and subsets of T lymphocytes [10,72,106,107]. JAM-L has been described to promote leukocyte adhesion to endothelial CAR and its function is regulated *in cis* by the activation state of the VLA-4 integrin which controls its dimerization state [106]. JAM-L is also involved in both, monocyte adhesion and migration *in vitro* across endothelial monolayers activated with TNF α [108]. Interestingly, CAR has been reported to colocalize and physically interact with JAM-C in testis, however, the involvement of this interaction in leukocyte TEM has not been yet described [109].

JAMs in leukocyte transepithelial migration

Neutrophils or polymorphonuclear neutrophils (PMNs) are the first immune cells to be recruited to the mucosal intestinal epithelium after their emigration from the local microvessels. In contrast to polarized transmigration in the apical to basolateral direction across endothelia, PMN migrate in the reverse direction across epithelia in a basolateral to apical or luminal direction (Figure 2, table 2). PMN transepithelial migration begins with initial adhesive interactions to the basal aspect of the epithelium involving the leukocyte integrin CD11b/CD18 and fucosylated counter ligand(s) not yet identified [110–112]. CD11b/CD18 is clearly a major PMN adhesive integrin mediating initial interactions with intestinal epithelia. However neutrophil transepithelial migration can occur in a CD11b/18-independent manner depending on the chemoattractant signal and type of epithelium [113–

115]. After initial adhesion, PMN negotiate the paracellular space along the basolateral epithelial membrane through interactions with CD47, a transmembrane glycoprotein that binds to its receptor on PMN termed SIRP α (Signal Regulatory Protein alpha) [116–118]. CD47 and SIRP α binding interactions have also been shown to regulate the rate of PMN migration across the endothelium [119,120]. As PMNs migrate across epithelial intercellular junctions, they disrupt E-cadherin-mediated cell contacts in an elastase-dependent manner [121]. How neutrophils cross TJs is poorly understood. While direct interactions of PMN with structural components of epithelial TJs has not been shown, it is now appreciated that JAM related proteins expressed on neutrophils and in epithelial TJs play potentially important roles in regulating PMN transepithelial migration. In particular, CAR has been shown to mediate PMNs transepithelial migration through heterophilic interactions *in trans* with JAM-L on PMN [122]. Analogous experiments with antibodies that block barrier forming properties of JAM-A in epithelial cells do not inhibit neutrophil transendothelial or transepithelial migration [28]. The contribution of other related JAM family members such as JAM4 and CLMP, while uniquely positioned to participate in regulation of PMN transepithelial migration, have not yet been described in this process. Thus, the role of JAMs and related proteins in PMN transmigration remains poorly understood, but it has become increasingly apparent that these molecules have important signaling functions that may indirectly regulate leukocyte trafficking. For example, transepithelial migration process requires transient disruption of the intercellular junctional complexes and it is now apparent that JAM members play an important role in regulation of epithelial barrier. These observations raise the possibility that epithelial JAMs, instead of functioning as leukocyte counter receptors, may be involved in highly regulated signaling pathways that control the transient opening and closing of the epithelial barrier during the transmigration response [123–125]. After transepithelial migration, PMNs arrive at the apical or luminal epithelial surface where they remain in close apposition with epithelial cells. Under inflammatory conditions, it is now appreciated that epithelial cells upregulate ligands for PMN β 2 integrins such as ICAM-1 on the apical or luminal epithelial membrane [126,127]. In recent studies, we have observed that apically expressed epithelial ICAM-1 may actually facilitate neutrophil recruitment across the intestinal epithelium through signaling events that increase permeability by myosin light chain kinase-dependent contraction of the perijunctional actomyosin ring [128]. PMNs have also been shown to interact with other apically expressed epithelial ligands such as the V6 variant of CD44 resulting in its shedding and subsequent detachment of PMNs from the epithelium [129]. CD55 (Decay accelerating factor) has been also implicated in the neutrophil detachment from the apical surface [130]; however the leukocyte counter receptors of these anti-adhesive epithelial molecules (CD44v6 and CD55) have not been identified.

The yin and the yang of JAMs in the inflammatory response

Under normal conditions after mucosal injury, it is now well appreciated that PMNs, in addition to their antimicrobial function that is associated with tissue damage, promote wound healing and tissue repair by the secretion of pro-resolution factors (resolvins, lipoxins and protectins). PMNs also trigger the recruitment of monocytes that phagocytose dead/dying neutrophils to efficiently remove potentially tissue damaging components [131–133]. There is now strong evidence for a role of JAM proteins in epithelial repair responses. Other

leukocytes that reside in intimate contact underneath and between epithelial cells termed $\gamma\delta$ T lymphocytes ($\gamma\delta$ -IELs) have been shown to play important roles in epithelial healing. These repair events involve production of growth factors such as keratinocyte growth factor (KGF) also known as fibroblast growth factor 7 (FGF7) that promotes cell proliferation and restoration of barrier function [134,135]. Indeed, $\gamma\delta$ -IELs in murine skin express JAM-L and its interaction with CAR expressed on keratinocytes has been shown to act as a costimulatory signal resulting in increased T-cell proliferation, production of cytokines and growth factors which enhance dermal wound healing [136]. In line with a possible positive role for $\gamma\delta$ -IELs and JAM-L in intestinal mucosal recovery after injury, $\gamma\delta$ -IELs have been found in large numbers within areas of mucosal damage in a DSS-colitis murine model. In addition, $\gamma\delta$ -IELs-deficient mice or KGF-deficient mice are more susceptible to DSS-induced mucosal injury and have delayed tissue repair. These findings strongly suggest that $\gamma\delta$ -IELs may promote intestinal mucosal wound healing after injury [137]. In contrast to a pro-resolving role of JAM-L in $\gamma\delta$ -IEL function, recent investigations on JAM-L in neutrophils have suggested pro-inflammatory functions. In particular, it was reported that JAM-L is cleaved from neutrophils in a zinc-metalloprotease-dependent fashion during neutrophil transepithelial migration, and the soluble JAM-L ectodomains bind to epithelial CAR resulting in delayed recovery of barrier and inhibition of wound repair through downregulation of epithelial cell proliferation ([138]). These observations suggest that JAM-L released from migrating PMN during acute mucosal inflammation has a pro-inflammatory role to facilitate further recruitment of leukocytes by inhibiting barrier function as well as wound healing. It is tempting to speculate that JAM-L release from activated PMNs may be partly responsible for poorly healing of mucosal ulcers associated with massive leukocyte infiltration as observed in inflammatory bowel disease (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD) [139,140]. In an analogous fashion, JAM-A and JAM-C have been reported to be cleaved from endothelial cells under inflammatory conditions. Proinflammatory cytokines (TNF- α and IFN- γ) induce the cleavage of the extracellular region of JAM-A and JAM-C by the disintegrin and metalloproteinases 10 and 17 (ADAM10/17) in endothelial cells. Soluble JAM-A ectodomains reduced transendothelial migration of neutrophils and endothelial cell migration [141] while soluble JAM-C promoted angiogenesis [142]. Interestingly, ADAM17 is upregulated by TNF α in the intestinal epithelium [143], and JAM-A has been reported to be released from epithelial cells [141]. Consequently, during inflammation, soluble JAM-A, JAM-C and JAM-L may be released in the intestinal mucosa from both, the epithelium and leukocytes to mediate distinct roles on leukocyte recruitment and mucosal repair. Further investigations are required to clarify the role of soluble JAMs in the intestinal mucosa during inflammation and repair.

Epithelial wound healing is a central component of mucosal homeostasis that depends on coordinated regulation of cell proliferation and migration. Among the JAM family members, a role for JAM-A in regulation of epithelial cell proliferation has been intensively studied. The loss of JAM-A (JAM-A deficient mice or *in vitro* siRNA depletion) has been shown to result in enhanced intestinal epithelial cell proliferation [31,32,144]. The presence of JAM-A has been reported to restrict intestinal epithelial-cell proliferation by negatively regulating the activation of Akt/ β -catenin. Conversely, the loss of JAM-A promoted phosphorylation of

Akt and activation of β -catenin resulting in nuclear translocation and enhanced transcriptional activity. Interestingly, in rescue experiments, only full-length JAM-A and not dimerization-defective JAM-A was shown to attenuate increased cell proliferation in JAM-A deficient cells, strongly suggesting that JAM-A dimerization and subsequent interaction *in trans*, are required to inhibit epithelial cell proliferation [144]. Thus, JAM-A signaling seems to downregulate Akt-dependent pro-proliferative signals. Such conditions are likely relevant during chronic intestinal inflammation since JAM-A expression has been found to be significantly reduced in the intestinal mucosa of individuals with IBD [32,145,146]. Analogously, JAM-A is also redistributed away from the TJ in the small intestine epithelium of a murine model of T cell-mediated acute colitis [147], presumably resulting in a loss of signaling function. A central observation in many chronic mucosal inflammatory conditions is dysregulated secretion of inflammatory mediators that has been directly linked to increased intestinal permeability and pathologic accumulation of leukocytes in the mucosal epithelium [148–151]. Among the pro-inflammatory cytokines, TNF α and IFN γ have been reported to be increased in the intestinal mucosa of IBD patients [152–154] and have been shown to have potent effect on barrier function by inducing disassembly of TJs, and redistribution of TJ proteins in epithelial cells [145,155–158]. IFN- γ for example has been reported to induce specific redistribution of JAM-A as well as occludin, claudin-1 and -4 from the lateral TJ membrane resulting in increased epithelial paracellular permeability [155]. IFN- γ -induced internalization of JAM-A and occludin involves a macropinocytosis-like process through RhoA/ROCK signaling [145,157]. Overall, the redistribution and/or internalization of JAM-A during inflammation may mimic a JAM-A deficient state inducing cell proliferation signals to repair the injured epithelium.

Among the JAM related members expressed on epithelial cells, CAR may be involved in epithelial proliferation and wound healing. Despite controversy, loss of CAR has been reported to promote the proliferation, migration and cancer progression [159,160]. In addition, CAR expression has been shown to be downregulated by pro-inflammatory cytokines in endothelial and epithelial cells [107]. However, the impact of inflammation on CAR expression in the intestinal mucosa is not well understood and needs further study. Nevertheless and already mentioned above, CAR has been reported to regulate epithelial intercellular junctions by promoting the internalization of E-cadherin [64,65]. Since cell surface E-cadherin associates with the transcription factor β -catenin, the internalization of E-cadherin by CAR may dissociate the E-cadherin- β -catenin complex allowing for β -catenin translocation into the nucleus resulting in enhanced cell proliferation. In line with this hypothesis, knockdown of CAR has been reported to downregulate α -catenin in a human colon cancer cell line, suggesting that CAR may regulate adherens junction proteins to control cell proliferation [161].

In addition to proliferation, a critical component of the mucosal wound healing process is cell migration, and many studies have linked JAMs to regulation of cell motility through integrins that mediate cell adhesion and migration. JAM-C dephosphorylation at Serine 281 has been reported to increase cell adhesion and migration by activation of β 3 integrins and deactivation of β 1 integrins [162]. JAM-A interacts *in cis* with α v β 3 integrin and dissociation of these proteins in response of bFGF promotes α v β 3-dependent endothelial cell

migration and angiogenesis [163,164]. JAM-A has also been shown to regulate cellular levels of $\beta 1$ integrin, and inhibition of JAM-A (function blocking antibody or downregulation) reduced the levels of $\beta 1$ integrin and cell migration in breast cancer cells [165]. The absence of JAM-A in neutrophils inhibits directional motility of cells toward a chemotactic stimulus by preventing $\beta 1$ integrins recycling during migration [85]. In epithelial cells, knockdown of JAM-A by siRNA, expression of JAM-A mutant proteins lacking the distal Ig-like loop or the dimerization motif was shown to decrease $\beta 1$ integrin staining at the cell surface, cell-matrix adhesion and cell migration [166,167]. JAM-A depletion or overexpression of cis-dimerization mutants decreased the active levels of the small GTPase Rap1, well-known to regulate integrin-mediated cell adhesion [166,167]. JAM-A has also been reported to interact with afadin and the guanine nucleotide exchange factor PDZ-GEF2, and the loss of JAM-A, Afadin, or PDZ-GEF2 decreased cellular levels of activated Rap1, $\beta 1$ integrin protein, and epithelial cell migration. Altogether, these findings describe a molecular pathway in which JAM-A cis-homodimers facilitate formation of a complex with Afadin and PDZ-GEF2 that activates the small GTPase Rap1A, which in turn promotes $\beta 1$ integrin stability and enhanced cell migration [166]. These results highlight a dual function of JAM-A-dependent signaling pathways in which JAM-A expression/dimerization is necessary for cell motility and polarization while suppressing pro-proliferative signals.

Given the above highlighted roles of JAM-A in regulation of intestinal mucosal homeostasis, it is surprising that JAM-A knockout mice do not develop spontaneous intestinal inflammation [31,32]. When JAM-A-deficient mice are subjected to DSS-induced colonic epithelial injury, the lack of JAM-A results in significantly enhanced disease suggesting that JAM-A plays an important protective role in the gut. It is now appreciated that there are remarkable compensatory changes that occur secondary to loss of JAM-A. To compensate for the leaky epithelial barrier and enhanced exposure to luminal microflora, JAM-A-null mice have increased numbers of mucosal TGF- β producing CD4⁺ T cell subsets and mucosal plasma cells that produce increased levels of IgA [168]. These findings indicate a crucial protective role of the adaptive immune system under conditions of chronically increased intestinal permeability and emphasizes the complexity mucosal homeostasis that is dependent on multiple cellular constituents which act in concert to prevent disease.

Conclusion

As highlighted in figure 2, we have discussed a multitude of experimental observations on JAMs and related family members as they relate to mucosal function during homeostasis and inflammatory disease. There is now strong evidence that JAMs play an important role in regulating paracellular permeability of both epithelia and endothelia and their loss or redistribution during chronic inflammation may worsen the progression of disease as reported for JAM-A in murine models of IBD ([31,32]). During inflammation, the release of proinflammatory cytokines such as IFN γ and TNF α results in altered and/or reduced expression of certain JAMs and disruption of JAM-dependent signaling pathways. Consequently, a better understanding of the mechanisms responsible for the modulation of JAMs expression in mucosa during inflammation and following anti-inflammatory therapies

may help to design new strategies to strengthen the epithelial barrier and prevent abnormal leukocyte recruitment.

In addition to epithelial paracellular permeability, it is now clear that JAMs are important regulators of cell proliferation and migration. While certain JAMs have been shown to play important roles in epithelial wound healing, enhanced expression of JAM-A, -C and CAR has also been associated with worse clinical progression of various epithelial cancers [159,160,162,165,169,170]. Consequently, the targeting of JAM members may have clinical implications in reducing cancer progression. Along similar lines, since JAM-A and CAR are receptors for reovirus and adenovirus respectively, unique opportunities for virus-based anti-tumoral drug delivery may be afforded. In conclusion, JAM family members are expressed in cells that play critical roles in regulation of mucosal homeostasis. Better understanding the cell-type dependent functions of JAMs will thus offer new insights into the pathobiology of inflammatory mucosal diseases and ideas for new therapeutics.

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Figure 1. Intestinal mucosa barrier

a) Hematoxylin-Eosin staining of mucosal mouse colon. The mucosa is composed of a single layer of columnar epithelial cells that are separated from luminal contents by a mucus. The epithelium includes enterocytes (absorptive cells with microvilli), goblet cells that secrete mucus, and the lamina propria containing immune cells, capillaries, lymphatic vessels. **b)** Molecular organization of the epithelial intercellular junctional complexes: Tight junction (TJ), subjacent adherent junctions (AJs) and desmosomes (DE). TJ and AJ are collectively referred to as the Apical junctional Complex that associates with an underlying perijunctional actin-myosin ring. TJ contains three transmembrane proteins: Occludin, Claudins and JAMs that bind to numerous TJ-associated scaffold molecules, such as Zonula occludens proteins (ZO). JAM, Junctional Adhesion Molecule; CAR, Coxsackie and Adenovirus Receptor; CLMP, CAR-Like Membrane Protein.

Figure 2. Proposed roles of JAM proteins in mucosal homeostasis and inflammation

a) Homeostasis of the epithelial barrier. JAM-A has been shown to regulate epithelial barrier paracellular permeability, cell polarity, migration and proliferation through the activation of distinct molecular pathways. Dimerized JAM-A associates with distinct signaling scaffolds highlighted by different colored circles. For barrier function, JAM-A association with afadin/PDZ-GEF1/Rap2c results in inhibition of RhoA and pMLC to regulate tension of the perijunctional actomyosin ring. In addition JAM-A recruits the polarity complex Par-3/Par-6/aPKC to maintain epithelial cell polarity. JAM-A keeps proliferation in check by inhibiting the phosphorylation of AKT through an as yet unidentified process. For cell migration, JAM-A association with afadin/PDZ-GEF2/Rap1A serves to stabilize β 1 integrin expression and localization. **b) PMN migration out of blood vessels and across the epithelium during acute inflammation.** Migration of neutrophils out of the circulation and across the epithelia is a multistep process involving many adhesion molecules and different JAMs (table 2). In addition to playing a role in neutrophils adhesive interactions with CAR, cleavage of JAM-L may have pro-inflammatory properties by inhibiting barrier recovery during the transmigration response. **c) The epithelial barrier during chronic inflammation.** Under chronic disease conditions associated with increased levels of inflammatory cytokines, the loss/redistribution of tight junction proteins including JAM-A results in dysregulated signaling pathways highlighted above in (a) causing increased proliferation, enhanced permeability through contraction of the actomyosin ring and decreased cell migration. Akt/Protein kinase B; β cat, b-catenin; CAR, Coxsackie and Adenovirus Receptor; CAMs, Cell Adhesion Molecules; (PECAM, ICAM-1/2, VCAM); JAM, Junctional Adhesion Molecule; Par, Partitioning-defective protein; SIRPa, Signal Regulatory Protein alpha; pMLC, phosphorylated Myosin Light Chain protein; sJAM-L, soluble JAM-L.

Table 1

JAM and related proteins: tissue distribution and main functions.

Molecule	Tissue distribution	Cells	Associated Function	References
JAM-A	Blood, Bone marrow, Brain Heart, Intestine Kidney, Liver Lung, Pancreas Skin, Spleen Testis	Endothelia Epithelia M, N, L, DC, Pl, MΦ Spermatozoa/Sertoli cells HSC	Barrier Permeability TEM/TEpM Angiogenesis Proliferation Cell migration Spermatogenesis Self-renewal	[2,3,15,28,45,85, 86,144,171–174]
JAM-B	Heart, Kidney Lymph nodes Intestine, Testis	HEV Lymphatics Sertoli cells HSC, NSC, ESC	Barrier Permeability TEM	[5,71,88,172, 175]
JAM-C	Brain, Bone marrow, Heart, Lung, Liver, Kidney, Spleen, Testis Nerve	M, L, DC, Pl, NK HEV Lymphatics Spermatid HSC Schwann cells Fibroblast	Barrier Permeability TEM (Reverse migration) Angiogenesis Cell differentiation self-renewal Nerve conduction	[7,71,87,92, 172,176–179]
JAM4	Blood, Bone marrow, Intestine, Kidney, Liver, Testis	Epithelia Spermatogonia HSC	Barrier Permeability	[9,55,180]
JAM-L	Blood Skin	M, N, L γδIEL	Cell Adhesion TEM/TEpM Cell activation Wound repair	[106,108,122, 136]
CAR	Brain, Kidney, Liver, Lung, Intestine, Skin Testis	Endothelia Epithelia Lymphatics Spermatozoa	Barrier Permeability TEM TEpM	[57,60,72,122, 136,181]
CLMP	Adipose tissue, Brain, Heart Intestine, Kidney Lung, Testis	Epithelia	Development Adipocyte maturation	[12,67,182]
ESAM	Bone Marrow, Heart, Lung, Liver, Lymph nodes	Platelet HEV Lymphatics HSC	Barrier Permeability TEM Angiogenesis self-renewal	[13,74,103,183, 184]

JAM, Junctional Adhesion Molecule; JAM-L, Junctional Adhesion Molecule-Like protein; CAR, Coxsackie and Adenovirus Receptor; CLMP, CAR-Like Membrane Protein, ESAM, Endothelial-Selective Adhesion Molecule; HSC, hematopoietic stem cells; NSC, Neural stem cells; ESC, embryonic stem cells; M, Monocyte; N, Neutrophil; L, Lymphocyte; DC, Dendritic cell; Pl, Platelet; MΦ, Macrophage; NK, Natural killer; HEV, High Endothelial Veinule; TEM, Transendothelial migration; TEpM, Transepithelial migration; IEL, intraepithelial lymphocyte.

Table 2

Comparison of molecular interactions involved in leukocyte migration across endothelia versus epithelia.

Leukocyte Transendothelial migration			Leukocyte Transepithelial migration		
Luminal $\Rightarrow$ AbLuminal			AbLuminal $\Rightarrow$ Luminal		
STEPS	Endothelial cell	Lymphocyte, Monocyte, Neutrophil	STEPS	Epithelial cell	Neutrophil
Tethering	P-Selectin E-Selectin PSGL-1 VCAM-1 MADCAM1	PSGL-1 PSGL-1/CD44/ESL-1 L-Selectin VLA-4 (CD49d/CD29) $\alpha 4\beta 7$	Initial contact	Fucosylated proteins	Mac-1 (CD11b/CD18)
Rolling	E-Selectin ICAM-1 VCAM-1	PSGL-1/CD44/ESGL-1 LFA-1 (CD11a/CD18) VLA-4	Basolateral membrane	CD47	SIRP α
Firm Adhesion	ICAM-1 ICAM-2 VCAM-1 CAR	LFA-1/Mac1 LFA-1/Mac1 VLA-4 JAM-L	AJs	Loss of E-cadherin based contacts	Proteases Elastase
Crawling	ICAM-1 (ICAM-2)	Mac1 (LFA-1)	TJs	CAR	JAM-L
Diapedesis	ICAM-2 JAM-A JAM-B JAM-C CAR ESAM PECAM-1 CD99 CD47	LFA-1/Mac-1 LFA-1/JAM-A VLA-4/JAM-C Mac1/JAM-C JAM-L ? PECAM-1 ? SIRP α	Luminal interaction	ICAM-1	Mac-1
Basal Lamina	Collagen IV Laminins (low density)	Proteases, MMPs $\alpha 6\beta 1$ -integrin	Detachment	CD44V6 CD55	? ?

Coxsackie and Adenovirus Receptor (CAR); E selectin ligand 1 (ESL1); endothelial cell-selective adhesion molecule (ESAM); intercellular adhesion molecule 1/2 (ICAM1/2), junctional adhesion molecule (JAM); Junctional Adhesion Molecule-Like protein (JAM-L); lymphocyte function-associated antigen 1 (LFA-1); macrophage antigen 1 (MAC1); mucosal vascular addressin cell adhesion molecule 1 (MADCAM1); matrix metalloproteinases (MMPs); platelet endothelial-cell adhesion molecule-1 (PECAM-1); P selectin glycoprotein ligand 1 (PSGL1); vascular cell-adhesion molecule 1 (VCAM1); very late antigen 4 (VLA4). See for review [70,132].