JAM Family and Related Proteins in Leukocyte Migration (Vestweber Series)

Paul F. Bradfield, Sussan Nourshargh, Michel Aurrand-Lions, Beat A. Imhof

Abstract—Exploring the role of junctional adhesion molecules (JAMs) has proven to be varied and controversial. The purpose of this review is to discuss the new and exciting roles of these IgSF molecules and how they have evolved to contribute to diverse functions from development to inflammation. In particular, recent research has focused on JAM subfamily members JAM-A, -B, and -C with newly described roles in leukocyte trafficking during inflammation and angiogenesis. However, research on all JAM family members has demonstrated recurring themes with striking similarities in the many diverse processes they are now known to regulate. (Arterioscler Thromb Vasc Biol. 2007;27:2104-2112.)

Key Words: endothelium ■ leukocyte ■ transmigration ■ inflammation

Understanding how leukocytes are targeted from the vasculature to sites of inflammation has been an active area of investigation over the last 20 years. It occurs in a multistep adhesion cascade involving the sequential engagement of adhesion and signaling receptors in a combinatorial process under shear stress, culminating in transendothelial migration.1–3 Leukocyte trafficking plays an integral role in immune surveillance, a process that requires leukocytes to transcend endothelial barriers between different tissue compartments, optimizing relevant cell interactions and facilitating recirculation.4–6

Leukocytes can exit the vasculature by movement through the body of endothelial cells, although extravasation primarily occurs by migration via junctions between adjoining endothelial cells.7–10 This involves multiple interactions, as the endothelium possesses a network of proteins that serve as barriers preventing solutes leaking from the vasculature, and preserve cell polarity by maintaining a molecular boundary between the luminal and abluminal membrane domains.11 Although interactions between leukocytes and endothelial cells during the initial phases of adhesion under luminal shear stress continues to be an area of interest, the complex interplay of molecular interactions at the interendothelial junction during the latter phases of adhesion, particularly transmigration, has become a major focus of research.12 Particular attention has been paid to molecules such as CD99, VE-Cadherin, PECAM-1, and the junctional adhesion molecules (JAMs) that are all distributed at endothelial junctions, where cis and trans-interactions enable endothelial cells to interact with and regulate leukocyte migration into underlying tissues.13

Origins of JAMs: A Relic or Regulator of the Immune Response?

JAMs are extended members of the CTX (Cortical Thymocyte marker for Xenopus) family and are a group of proteins...
with a V-C2 IgSF type structure. These proteins are unusual as they have clearly identifiable cell receptor homologues in many primitive invertebrates (eg, protochordates). The existence of the genes encoding CTX family members on multiple chromosomes suggests that they derive from multiple duplication and translocation events creating paralogous copies. Phylogenetic studies have sparked great interest in the CTX family, as ancestral precursors of this family may be the origin of B-cell and T-cell immune receptors. However, many of the CTX molecules have been characterized as receptors for a broad range of viruses. Although viruses can use a wide array of cell receptors to gain entry to a cell, it has been shown that engagement of JAM-A by a particular virus can specifically trigger apoptosis. Such a precise mechanism has led to speculation that this may be a relic of a putative ancestral receptor mediating a suicide immune response to minimize infection, ie, containment to prevent propagation of an infectious virus. The development of such a primitive innate immune response would require the expansion of the core V-C2 gene complex, driven by an ever-increasing repertoire of viruses. This may have been a critical event in the evolution of an effective adaptive immune response, with an ever-expanding reservoir of V-C2 gene complexes and recombination between each other.

In conclusion, multiplicity in CTX functions has suggested that they have evolved from a single ancestral gene with a comparatively primitive immune function, into a distinct group of molecules that participate in the complex interplay of the innate and adaptive immune response. Thus, CTX molecules may have played a critical role in shaping the human immune system.

### The Junctional Adhesion Molecule Family

Junctional adhesion molecules (JAMs) encompass a family of 3 closely related CTX proteins and 4 members of another CTX subfamily with described functions; the classical JAMs are JAM-A, JAM-B, and JAM-C, the other family members are ESAM, CAR, JAM-4, and JAML (Table). They comprise the 2 extracellular Ig domains with either a short or a long cytoplasmic domain, respectively, containing PDZ binding domain of type I or type II (except for JAML), and multiple phosphorylation sites (Figure 1).

#### Class I PDZ Binding Domain

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<th>Class</th>
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<td>I</td>
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#### Class II PDZ Binding Domain

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<td>Maintenance of endothelial tight junctions mediated by interactions with JAM-A by a particular virus can specifically trigger apoptosis.&lt;sup&gt;19&lt;/sup&gt;</td>
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#### Classical JAM Proteins

Perhaps the best characterized members of the JAM family are JAM-A, -B, and -C. Differential expression or redistribution of these molecules at endothelial cell junctions in certain inflammatory scenarios contribute to leukocyte interactions and trafficking.<sup>23,24</sup> JAM-A was originally characterized as the human platelet F11 receptor<sup>25,26</sup> before being cloned and identified as JAM. Crystallography studies of JAM-A structure have revealed that U-shaped JAM-A dimers formed in cis can interact in trans with JAM-A dimers expressed on opposing endothelial cell junctions. However, it has been suggested that during leukocyte transendothelial migration, such homophilic interaction of JAM-A may be replaced with heterophilic trans-interactions involving leukocyte integrin counter-receptors.<sup>22,24</sup> In line with this, the integrin LFA-1 (α<sub>L</sub>β<sub>2</sub>, CD11c/CD18) has been identified as a ligand for JAM-A, an interaction that reportedly mediates leukocyte transmigration through cultured endothelial cells and also contributes to their...
adhesion under conditions where JAM-A is mobilized to the luminal surface of the endothelium.31 Although more recent studies have confirmed that during leukocyte transmigration JAM-A and intercellular adhesion molecule-1 (ICAM-1) can redistribute on the endothelial cell surface and colocalize with LFA-1 within ring-like clusters on adherent neutrophils,32 obtaining additional evidence for a direct JAM-A/LFA-1 interaction has proved elusive. JAM-A is also capable of forming cis-interactions with integrin ligands. It interacts with the integrin \( \beta_1/\alpha_4 \) on endothelial cells, a cis-interaction that has been implicated in regulating cell migration through the MAPK pathway.33 However, the role of JAM-A in regulating \( \beta_1/\alpha_4 \) dependent migration on leukocytes remains unknown. Other studies have proposed that JAM-A may primarily function as a regulator of polarity, playing a critical role in assembly and remodeling of retinal pigment epithelial tight junctions.34 Interestingly, this function has been extended to polymorphonuclear leukocytes (PMNs) during inflammation.35,36 In this study JAM-A–deficient neutrophils were found to be defective in terms of their polarized movement.35 JAM-A–deficient mice have also provided conclusive evidence for the involvement of JAM-A (leukocyte or endothelial) in leukocyte migration into sites of inflammation as studied in models of peritonitis, myocardial ischemia reperfusion, hepatic ischemia reperfusion, and a model of vascular injury in atherosclerosis-prone mice.35–37 In the latter study, the interaction of monocytes with carotid arteries subjected to wire injury was also analyzed ex vivo and JAM-A deficiency was found to be associated with reduced leukocyte arrest on denuded vessel segments.36 Details of the mechanisms that mediate these events is at present unclear, but JAM-A appears to be upregulated on endothelium of carotid arteries in atherosclerotic prone mice.38 In addition, the enhanced luminal expression of the chemokine RANTES in injured arteries in vivo and its endothelial cell deposition by activated platelets in vitro appears to be JAM-A–dependent.36,37 Together the findings of these studies indicate that depending on the nature or temporal phase of the inflammatory reaction, expression of JAM-A on leukocytes, platelets, and endothelial cells can have a significant role in mediating leukocyte attachment or transmigration in vivo. For JAM-B and JAM-C the situation is even more complex.

JAM-B and JAM-C interact with each other and can form multimer interactions with integrin counter-receptors. JAM-B can bind the integrin VLA-4 (\( \beta_1/\alpha_4 \), CD29a/CD49d), but requires the presence of JAM-C on the \( \beta_1/\alpha_4 \) integrin expressing cells,39 whereas JAM-C can act as a counter-receptor for the integrin Mac-1 (\( \beta_2/\alpha_4 \), CD11c/CD18) (Figure 2).40 Furthermore, an extended role for JAM-C as the leukocyte counter receptor for JAM-B expressed on endothelial cells has been described on human circulating platelets, NK cells, dendritic cells, and subsets of T and B cells.23,39,41–44 As discussed, JAM-B and -C have been implicated in many diverse biological processes such as angiogenesis,45 leukocyte migration,43,46–48 and spermatogenesis.49 Particular attention has focused on the role of JAM-B and -C in leukocyte accumulation during inflammation.48,50,51 Initial studies showed that functional blocking antibodies to JAM-C can reduce transmigration of peripheral blood lymphocytes across cultured HUVECs.43 Later studies identified Mac-1 as a ligand partner capable of forming trans-interactions with JAM-C that mediate adhesion and transmigration for neutrophils and monocytes,40–45,51,52 but uncertainty still exists about a specific lymphocyte ligand that interacts with endothelial JAM-C. A recent study proposed an extended role for JAM-C/Mac-1 interactions in regulating leukocyte function. Briefly, JAM-C expressed on adherent platelets was shown to

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**Figure 1.** Amino acid sequence alignment of cytoplasmic tails for mouse JAMs and JAM-related proteins. The cytoplasmic tails for JAMs are shorter (40 to 50 residues) than JAM-related proteins (105 to 120 residues), all ending in a PDZ binding domain22 with the exception of JAML. Direct comparison of JAM and JAM-related protein subfamilies is based on sequence alignment analysis conducted previously with JAM-B, ESAM, and CAR.71 Consensus between subfamily member sequences are marked in gray and indicated for both families separately. Comparison between sequences encompassing more than 1 subfamily are marked in boxes and shown as a master consensus.

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directly regulate dendritic cell activation via interaction with Mac-1, as well as mediating the initial adhesion events involved in recruitment from the vasculature.42

Deciphering the complexity of JAM-B/-C interactions with their respective ligands has proven particularly challenging and has been addressed in numerous studies. JAM-C as a functional ligand of JAM-B was described in vivo using a mouse model of allergic contact dermatitis, where a synergistic effect in blocking leukocyte accumulation at sites of inflammation is observed with combined blocking antibodies to JAM-B and JAM-C.53 JAM-C has been identified in desmosome structures on human epithelium, and it regulates neutrophil transepithelial migration by interacting with Mac-1 on neutrophils.52 This process can be further modulated by interactions with neutrophil JAML and epithelial CAR; another ligand for JAM-C.54 Similarly, blocking the JAM-B/-C interactions reduced neutrophil transepithelial migration under static conditions and reduced accumulation of PMNs within inflammatory lesions in vivo.55 However, in vitro studies conducted under physiological flow have given contradictory results. Neutrophil adhesion and transmigration on stimulated HUVECs under shear stress has been shown to be JAM-C–independent, with ICAM-1 identified as the preferred ligand for β2-integrins.55 This apparent contradiction may indicate a redundancy in JAM-C–mediated transmigration under certain conditions, as blocking JAM-C function had a small effect but only after blockade of other molecular pathways involved in leukocyte transmigration, ie, blockade of CD31- and CD99-mediated transmigration.55 This suggests that the consequence of JAM-C interactions with ligand partners differed under flow or static conditions, a phenomenon that could be attributable to differential JAM-C–triggered signaling events.

A link between JAMs and integrin function has also been identified by the small GTPase Rap1.56,57 Activation of Rap-1 forms a complex with the Rap1-ligand (RapL) and associates with integrins in focal adhesions.58,59 Blocking JAM-C function reduced permeability and neovascularization in vascular endothelial cells in mouse angiogenesis models of hypoxia.57

This mechanism is mediated by JAM-C regulating actomyosin-dependent contractility, and stabilization of VE-cadherin interendothelial cell contacts in a Rap1-dependent manner.57 This has proven particularly insightful as Rap1 has previously been shown to be involved in regulating endothelial barrier function.60 Later studies demonstrated that Rap1 activity played a pivotal role in modulating VE-cadherin trafficking and stabilization at endothelial cell junctions.61

The observation that reduced JAM-C expression increased Rap-1 activity and stabilization of the endothelial junction is intriguing as JAM-A is known to have an opposite effect in epithelial cells.56 This has led to the proposal that JAM-A functions as a junctional gatekeeper, whereas the presence of JAM-C promotes permeability.57 The fact that JAM-A and JAM-C have a high homology yet exert opposite regulatory effects is of particular interest considering that the evolution of these molecules occurred in parallel. The divergent roles of these molecules may be accounted for by variation in a single phosphorylation site (Figure 1). In addition to the above, the contribution of other heterophilic interactions at the tight junction must also be considered, particularly the role of JAM-B and CAR.

### JAM-B and JAM-C “Ying-Yang” Interactions at the Endothelial Junction

Accumulation of JAM-B and -C at the endothelial and epithelial intercellular junctions suggests that these molecules are involved in homophilic interactions. However, interpretation of these observations has proven to be difficult because of the multiple cis/trans heterophilic interactions that can occur (Figure 2). A good example of this was highlighted by a previous study that identified a hierarchy of JAM-B/C interactions occurring at cell junctions with low turnover for heterophilic and rapid turnover for homophilic interactions.47

Many in vitro studies conducted with primary cells and cell lines transfected with JAM-A, -B, and -C have appeared to be contradictory.51,55,62,63 For example, CHO cells transfected with JAM-C were shown to have increased paracellular permeability,62 whereas KLN cells transfected with JAM-C exhibited increased barrier function.63 Thus, cell functions regulated by JAM-B and -C may well be dictated by the ratio of ligand interactions, where an increase in JAM-C expression may shift to low affinity, homophilic interactions, and a
perceived increase of permeability of cell monolayers. This concept of disrupting the equilibrium of JAM-B/-C interactions has distinct implications for interpreting observations made in vitro. As a result, discrepancies reported for JAM-B/-C function, as studied using cell lines, may be attributable to relative expressions of homophilic and heterophilic ligands.

An Emerging Novel Function of JAM-C

The precise role of JAM-C in regulating leukocyte emigration continues to be an area of investigation. Because JAM-C is expressed at endothelial cell junctions, recent studies have focused on the role of vascular JAM-C in the regulation of leukocyte recruitment and retention during inflammation, in particular the role of JAM-C as a regulator of leukocyte ingress and egress. The latter was postulated as a potential novel role for JAM-C in regulation of leukocyte accumulation after the observations that JAM-C–deficient mice exhibited an increase in peripheral blood neutrophils after an inflammatory challenge. This finding was not a consequence of increased neutrophil replenishment from the animal’s bone marrow or other lymphatic tissues as assessed in a passive transfer study. Briefly, the intravenous injection of labeled wild-type neutrophils into JAM-C–deficient recipients also resulted in a high circulation level after an inflammatory challenge. Conventional models of increased neutrophil numbers in the blood during inflammation would predict this finding as a result of reduced recruitment. However, in vitro studies with cocultures of human peripheral blood neutrophils on activated endothelium under shear flow conditions have shown that neutrophil capture, firm adhesion, and transmigration is JAM-C–independent. In a separate coculture model, Bradfield et al have shown that blocking JAM-B/-C interactions reduced monocyte numbers in the abluminal compartment through increased reverse-transmigration rather than by reduced transmigration. These in vitro results were in line with in vivo findings showing that blockade of JAM-B/C interactions reduced the number of monocytes in inflamed tissues attributable to increasing numbers of reverse-transmigrated monocytes in the peripheral blood. Recent studies have shown that neutrophils can also reverse-transmigrate and that reverse-transmigrated cells have a reduced capacity for repeat-transmigration. This phenomenon was also observed in vivo using an inflammatory model in trans-lucid zebrafish.

The above observations have raised the possibility that JAM-C transgenic and knockout mice have altered populations of reverse-transmigrated leukocytes in their circulation. For JAM-C knockout mice, the expanded neutrophil population may be the product of increased reverse-transmigration of inflammatory neutrophils, exhibiting reduced transmigratory properties. Conversely, this may also explain how mice that overexpress endothelial JAM-C have leukocytes with an enhanced transmigratory potential, because of a reduced population of reverse-transmigrated leukocytes in the blood. In conclusion, monocytes and neutrophils have distinct adhesive and transmigratory properties, and future studies involving JAM-B and -C will require careful identification of leukocyte subsets, particularly during adhesion and transmigration studies.

Collectively, JAM-B/-C function on endothelial cells appears distinct from other currently known adhesion molecules implicated in the multistep adhesion cascade of leukocyte recruitment, and this may explain why blocking JAM-C function gives only a partial effect. Current models of JAM-C function would predict a complete ablation in immune function in the absence of JAM-C expression, because of absent leukocyte recruitment and trafficking. However, observations made with JAM-C knockout mice exhibit a phenotype consistent with a compromised immune response. A reduced immune response could partly be a result of a compromised immune surveillance, a process that confines leukocytes to different routes of cell movement. Many adhesion molecules and chemokines are known to define the strictly segregated compartments in lymphatic tissues and therefore have a critical role in regulating the movement of cells in and out of these areas. Under normal homeostasis, one function of JAM-B and -C at the endothelial barrier may therefore be to orchestrate direction of leukocyte trafficking, ensuring a unidirectional migration from the vasculature. This would suggest that the principle mechanism of JAM-B/-C function would be in regulating polarity, as opposed to contributing to adhesion events that create physical barriers.

JAM-C Expression on Leukocytes: A Junctional Affair?

The functional implications of JAM-C expression on human leukocytes remains intriguing. Recent reports have shown that JAM-C is expressed on dendritic cells, platelets, and subsets of T and B cells in humans. To date, however, JAM-C expression has not been reported on mouse leukocytes. The pan-expression of human JAMs across leukocyte and endothelial/epithelial populations has introduced a further level of complexity to interpreting a precise role for these molecules in leukocyte trafficking.

One emerging hypothesis is that JAM-C expression on human lymphocyte populations may facilitate the transmigration events involved in the constitutive trafficking of lymphocytes through the vasculature and the lymphatics. This is based on observations that blockade of JAM-C function using soluble JAM-C increased monocyte reverse- and repeat-transmigration on cultured endothelium. In reference to the above described “ying-yang” model, increased JAM-C interactions at the endothelial junction leads to increased permeability of the endothelial monolayer. This has distinct implications for the presentation of membrane bound JAM-C by trafficking lymphocytes at endothelial junctions. The expression of JAM-C on particular T and B cells may allow a single cell to open the endothelial junction by displacing JAM-B/C interactions with JAM-C/C interactions, facilitating bidirectional passage through multiple endothelial barriers.

The JAM-Related Proteins

Endothelial cell-selective adhesion molecule (ESAM) is a JAM-related molecule expressed by endothelial cells and platelets that mediates homophilic cell-cell aggregation.
Studies with ESAM-deficient mice demonstrated that ESAM plays a role in extravasation of neutrophils during early inflammatory reactions irrespective of the type of stimuli.72 This effect was not seen with T cell migration in an inflammatory model of cutaneous delayed type hypersensitivity using ESAM-deficient or antibody-treated mice.72,73 ESAM localizes to tight junctions of endothelial cells70 and has been shown to regulate vascular permeability in mouse inflammatory models and during intravenous injection of vascular endothelial growth factor.72 Accordingly, ESAM supports the activation of the GTP-ase Rho, known to be involved in the function of tight junctions and leukocyte transendothelial migration.72 These observations are consistent with a role for ESAM described in tumor angiogenesis.74 Recent reports described that ESAM can also anchor cytoplasmic MAGI-1 at epithelial/endothelial junctions by extracellular homophilic adhesion.75 This study has raised an interesting interpretation of JAM function, where extracellular interactions may merely serve to anchor intracellular binding complexes in a fixed position at the cell membrane in order to coordinate precise signals that regulate cellular position and polarity.

Attention has also focused on the molecule coxsackievirus and adenovirus receptor (CAR). This molecule has proven to be an attractive target for adenovirus based gene therapy, but the true function of CAR is only starting to emerge.76–77 CAR has been shown to play a crucial role in embryogenesis mediating survival signals between cardiomyocytes during heart development.78 Recent reports have highlighted the potential complexity of CAR function in homophilic interactions at intercellular junctions as well as heterophilic interactions with JAML and JAM-C (Figure 2).54,79 Interactions of CAR expressed at epithelial tight junctions with JAML on neutrophils have been shown to regulate transcellular migration.54 More complex cis- interactions have been described between CAR and JAM-C in the acrosomal region of spermatids, although the precise role of this interaction in spermatogenesis is unclear.79 An earlier study had shown that JAM-C plays a critical role in regulating polarization of spermatids during the maturation process, with JAM-B postulated as a ligand present on resident sertoli cells.49 However, as JAM-B deficient animals are fertile,80 CAR and JAM-C may be used as trans-interactors of JAM-C in Sertoli-germ cell junctions.79 These studies emphasize some of the potential complexities and problems associated with characterizing JAM functions, where multiple cis- and trans- interactions can occur that are not mutually exclusive (Figure 2).

JAM4 was originally characterized as an adhesion molecule that regulates permeability of epithelial cell monolayers in association with a membrane-associated guanylate kinase protein MAGI-1.81 Localization has been described at tight junctions in intestinal and kidney epithelial cells.81 Recent studies have also identified JAM4 expression on male germ progenitor cells and hemopoietic cell lineages,82 but the physiological role of JAM4 remains unclear as JAM4 knockout mice display no obvious functional phenotype.83 A role for JAM4 has however been suggested under pathological conditions in the kidney.83 Here, the authors were able to identify altered JAM4 expression and localization on injured podocytes in a disease model of nephropathy.83 Of importance, there exists no information to date describing a role for JAM4 in leukocyte transepithelial or transendothelial migration.

### Signaling Through JAM Receptors

The signaling properties of classical JAMs and related JAM family members have also proven diverse. Most JAM family members have an intracellular PDZ binding domain which can act as an anchor for scaffolding adaptor proteins that regulate cytoskeleton function, cell migration, integrin activation, and cell polarity (Figure 1).12,22,75,84–91 Phosphorylation at specific sites on the cytoplasmic tail may further contribute to regulation.65

These findings have expanded a potential role for JAMs. Transmigrating leukocytes can deliver intracellular signals to endothelial cells (outside-in signaling) that could alter the interaction of JAMs with associated adaptor proteins and disrupt polarity at the endothelial junction.92 This may prove particularly relevant with studies on ESAM or JAM-C that were unable to identify an extracellular ligand in regulating neutrophil and monocyte transmigration respectively.65,72 One interpretation of JAM function may be that direct extracellular interaction with known or unknown ligand partners is not required, as regulation occurs through intracellular cascades and the engagement/dissengagement of these molecules at the endothelial junction.

Mechanisms that define classical JAM function in generating and maintaining tight junctions have been shown in studies investigating association with cytoplasmic partners ZO-1, AF-6, Mupp-1, and PAR-3.22 The molecule PAR-3 has proved to be of particular interest as it exclusively binds to JAM-A, -B, and -C, playing a critical role in the establishment and maintenance of tight junctions in epithelial and endothelial cells.22,93–95 Recent studies have shown classical JAM interactions may have an extended range in regulating cell function by mediating multiple cis-interactions. Conformational changes in the integrin αβ, induced by engagement of ligand peptide sequences (RGD) has been shown to enhance cytoplasmic binding to JAM-A, which can activate the MAPK pathway leading to enhanced αβ-mediated migration.84 Phosphorylation sites on the cytoplasmic tail of JAM-C have been shown to modulate cell migration by regulating β integrin activation, although cis interactions that may modulate JAM-C function have not been shown.63 These studies have highlighted some of the potential difficulties in interpreting JAM function, as direct cis-interactions with integrin ligands can further augment integrin function at distal sites.

### Concluding Remarks

Recent studies looking at JAM interactions have identified multiple homophilic and heterophilic cis/trans-interactions. Further regulation of JAM function occurs by establishing extracellular/intracellular multi-protein complexes. A recurrent theme in the JAM family is the remarkable stability in expression levels of these molecules under different conditions, suggesting an integral role under homeostatic and
pathological conditions.68,83 This suggests that JAM function is mediated by localization within specific subcellular structures and different affinities to multiple ligands. Furthermore, canonical findings based on JAM-A suggest that differential JAM expression may be sufficient to imprint cell migration and polarization properties in a cell-autonomous manner.35,96,97 Recent studies have demonstrated this small family of molecules contain distinct multifunctional domains, mediating a wide range of functions that regulate polarity and permeability. Clearly polarity plays a critical role in defining microenvironments, and JAMs contribute to maintaining specialized tissue structures without the confines of physical barriers.

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**Disclosures**

None.

**References**


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