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Review

The role of junctional adhesion molecules in cell-cell interactions

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Summary. Cell-cell-interactions are important for the regulation of tissue integrity, the generation of barriers between different tissues and body compartments thereby providing an effective defence against toxic or pathogenic agents, as well as for the regulation of inflammatory cell recruitment. Intercellular interactions are regulated by adhesion receptors on adjacent cells which upon extracellular ligand binding mediate intracellular signals. In the vasculature, neighbouring endothelial cells interact with each other through various adhesion molecules leading to the generation of junctional complexes like tight junctions (TJs) and adherens junctions (AJs) which regulate both leukocyte endothelial interactions and paracellular permeability. In this context, emerging evidence points to the importance of the family of junctional adhesion molecules (JAMs), which are localized in tight junctions of endothelial and epithelial cells and are implicated in the regulation of both leukocyte extravasation as well as junction formation and permeability.

Key words: Junctions, JAM, Cell adhesion, Leukocyte transmigration, Inflammation, Permeability

Interendothelial junctions

Depending on the tissue/organ location of a particular vascular bed, endothelial cells possess a number of junctions that maintain vascular integrity and regulate permeability and leukocyte transmigration. At least three types of junctions have been described (Kemler, 1993; Dejana et al., 1995, 2001; Johnson-Leger et al., 2000): (i) Tight junctions (zonula occludens), the most apical junctions, which form a very close intercellular adhesive contact and which include three types of transmembrane proteins, occludin, claudins and junctional adhesion molecules (JAMs), which are linked intracellularly to cytoskelettal signaling molecules such as zonula occludens-1 (ZO-1) or cingulin. (ii) Adherens junctions (zonula adherens) formed by cadherins, i.e. transmembrane glycoproteins that promote calcium-dependent, homophilic cell-cell contacts. The link between cell-membrane-associated cadherins and the actin cytoskeleton is mediated by intracellular catenins. VE-cadherin is a member of this family specifically found in the inter-endothelial junctions. (iii) Gap junctions are clusters of transmembrane hydrophilic channels that do not serve a cellular contact function, but promote the exchange of ions and small molecules relevant for lateral transmission of molecular information. Here, we will focus on the family of JAMs, which are components of the tight junctions.

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Structure and cellular expression of JAMs

JAMs belong to the CD2 subgroup of the immunoglobulin superfamily, consisting of two extracellular Ig-like domains, a membrane-distal V-Ig-domain and a membrane-proximal C2-Ig-domain (Williams and Barclay, 1988; Barclay et al., 1997), followed by a single transmembrane region and a short cytoplasmic tail. Table 1 shows a synopsis of JAMs. The genes for JAM-A, JAM-B and JAM-C are localized on different chromosomes and the encoded mature glycoproteins have molecular masses of 30-43 kDa (Bazzoni, 2003; Chavakis et al., 2003). At their final carboxy-terminus (5 C-terminal amino acids) all three molecules have a class-II PDZ domain-binding motif, which predisposes them to interact with PDZ-domain-containing molecules, such as the ones found in tight junctions (Ebnet et al., 2004). The recently described JAM-4 differs from the three members of the JAM subfamily in its cytoplasmic tail. As opposed to 40-49 residues of other JAMs, the cytoplasmic domain of JAM-4 consists of 105 residues and its 5 C-terminal residues represent a type-I PDZ domain-binding motif (Hirabayashi et al., 2003). JAM-A, also referred to as JAM-1 or F11R (Kornecki et al., 1990; Naik et al., 1995; Sobocka et al., 2000) is found on different circulating

blood cells including platelets, monocytes, lymphocytes and erythrocytes as well as on endothelial and epithelial cells. The expression pattern of JAM-B (also called VE-JAM, human JAM-2, mouse JAM-3), which shares a 35% sequence identity with JAM-A is more restricted, as it localizes on vascular and lymphatic endothelium and especially at high endothelial venules, suggesting a special role for JAM-B in lymphocyte homing (Palmeri et al., 2000). JAM-C (also referred to as human JAM-3 and mouse JAM-2) is expressed on endothelial cells, on platelets (Aurrand-Lions et al., 2001a,b; Santoso et al., 2002) and on epithelial cells (Chavakis et al., unpublished observation). Moreover, human JAM-C is found on T-cells and natural killer cells (Liang et al., 2002; Santoso et al., 2002). Contrastingly, mouse T- and NK-cells do not seem to express JAM-C (Johnson-Leger et al., 2002).

Homophilic and heterophilic interactions of JAMs

Homophilic interactions: Implications for tight junction formation and regulation of paracellular permeability

The first evidence that JAMs could undergo a homophilic binding with JAM molecules came from transfection studies. In JAM-A-, -B, or –C-transfected cells the JAM molecules could be found at sites where transfected cells faced other transfected cells but not at sites where transfected cells contacted non-transfected cells (Martin-Padura et al., 1998; Cunningham et al., 2000; Aurrand-Lions et al., 2001a,b; Ebnet et al., 2001, 2003). At least for JAM-A such an interaction has also been shown biochemically. Soluble recombinant extracellular murine JAM-A interacts with itself, and in particular, extracellular soluble homodimers of JAM-A bind non-covalently to immobilized JAM-A homodimers (Bazzoni et al., 2000a). This homophilic interaction was prevented by monoclonal antibody against JAM-A, which recognizes dimeric rather than monomeric structures (Arrate et al., 2001). The N-terminal membrane-distal V-type Ig-like domain of JAM-A plays an important role in homophilic interactions of JAM-A (Babinska et al., 2002). Crystal structure analysis showed that JAM-A forms U-shaped homodimers *in cis* through a dimerization motif (RVE), which resides in the V_H fold (Kostrewa et al., 2001). This dimerization process seems to be a preceding step and structural prerequisite for homophilic interaction in trans (Kostrewa et al., 2001). In contrast, such homophilic interactions have not been described for JAM-B and JAM-C yet. Our preliminary observations indicate that such a potential interaction exists for JAM-C as well (Orlova et al., unpublished observations). However, as the dimerization motif is also present in JAM-B (RLE) and JAM-C (RIE), dimer formation is probable for JAM-B and JAM-C. Moreover, this common dimerization motif allows potential interactions amongst the different members of the JAM family or dimers thereof to be envisioned. In fact, JAM-B has been shown to interact with JAM-C (Liang et al., 2002), although the structural requirements of this interaction remain to be elucidated.

The homophilic interaction of JAM-A probably affects tight-junction formation, stability, and paracellular permeability, as inhibition of JAM-A with either blocking antibodies or soluble recombinant JAM-A protein resulted in a significant delay of the recovery of transepithelial electrical resistance in epithelial cells (Liang et al., 2000; Liu et al., 2000). Homophilic interactions between JAM family members might also contribute to cell-cell adhesion either in a homotypic (e.g. between adjacent endothelial cells) and/or a heterotypic manner (e.g. between leukocytes, endothelial cells, or platelets). However this has to be addressed in detail in future studies.

Ectopic expression of JAM-A or JAM-C in polarized epithelial cells results in their co-distribution with the TJ-marker protein ZO-1 suggesting direct association of both JAM molecules with TJs (Aurrand-Lions et al., 2001a). The subcellular localization of JAM-B is less clear because with ectopic expression in MDCK epithelial cells it is not specifically enriched at TJs (Aurrand-Lions et al., 2001b). A direct interaction with several PDZ-domain-containing molecules at tight junctions has been particularly shown for JAM-A: Such PDZ-domain molecules include ZO-1 (Bazzoni et al., 2000b), AF6/afadin (Yamamoto et al., 1997; Ebnet et al., 2000), MUPP 1 (multi-PDZ-domain-protein 1) (Hamazaki et al., 2002), and PAR-3 (partitioning defective), which forms a complex with atypical protein kinase C and PAR-6 (Ebnet et al., 2001; Joberty et al., 2000; Johansson et al., 2000; Lin et al., 2000; Qiu et al.,

Table 1. The JAM family members and their molecular mass, chromosomal localization and their homology to the murine protein. JAMs end in a type-II PDZ-domain-binding motif. X: position of the amino acids with respect to X0, which indicates the C-terminal amino acid.

PROTEIN	MOLECULAR MASS (kDa)	CHROMOSOMAL LOCALIZATION	HOMOLOGY TO MURINE PROTEIN	PDZ-DOMAIN-BINDING MOTIFS				
				X-4	X-3	X-2	X-1	X0
JAM-A	32	1	67%	S	S	F	L	V
JAM-B	40	21	79%	K	S	F	I	I
JAM-C	43	11	86%	S	S	F	V	I

2000; Suzuki et al., 2001). In the case of JAM-C, its association with ZO-1 is dependent on the phosphorylation status of the molecule, in particular, the interaction of JAM-C with ZO-1 is reciprocally dependent on the phosphorylation of the Ser281 in JAM-C (Ebnet et al., 2003).

Besides JAM family members, ZO-1 interacts with diverse cytoplasmic molecules, such as ZO-2 (Fanning et al., 1998; Wittchen et al., 1999), ZO-3 (Haskins et al., 1998), ß-catenin (Rajasekaran et al., 1996), heterotrimeric G-proteins (Saha et al., 2001; Meyer et al., 2002, 2003) and transmembrane proteins, such as occludin (Rajasekaran et al., 1996; Wittchen et al., 1999) and claudins (Itoh et al., 1999). Thus, JAMs may be involved in a multi-protein complex located at TJs. Moreover, ZO-1 and AF-6 are directly linked to the actin cytoskeleton (Fanning et al., 1998; Boettner et al., 2000).Therefore, a dynamic model of junction assembly, in which formation of complexes between JAM family members and ZO-1 or AF-6, which are then progressively recruited into more insoluble structures linked to the actin cytoskeleton, can be envisioned. Besides anchoring to the cytoskeleton, binding to AF-6 may enable JAMs to interact with regulatory molecules, as AF-6 is a target for the small GTPase Ras (Kuriyama et al., 1996). Activated Ras has been described to inhibit the interaction between AF-6 and ZO-1 (Yamamoto et al., 1997), and the interaction between AF-6 and JAMs might be involved in the Ras-induced regulation of cell contacts.

In addition, the PAR-3-αPKC-PAR-6 complex is essential for epithelial TJ formation, as its absence in cells which are in the process of developing cell polarity, leads to a mislocalization of several TJ-associated proteins resulting in changes in the TJ-function, e.g. reduction of transepithelial electrical resistance, which thereby increases paracellular permeability (Suzuki et al, 2001; Gao et al., 2002). Ectopic expression of JAM-A in CHO cells recruits PAR-3 to cell-cell contacts via the first three PAR-3 PDZ domains. Overexpression of a dominant-negative JAM-A mutant results in a PAR-3 dislocation from cell-cell contacts (Ebnet et al., 2001). Thus, JAM-A can actively recruit endogenous PAR-3 thereby regulating its function in paracellular permeability. This is in accordance with the observation that ectopic JAM-A expression in CHO cells decreases paracellular permeability. Interestingly, although JAM-C interacts with ZO-1 and PAR-3, its ectopic expression leads to an increase in cell permeability (Aurrand-Lions et al., 2001b). Here, the exact molecular mechanisms underlying these differences need to be addressed in detail in future studies. Such studies will provide new insights into the regulation of vascular permeability.

Heterophilic interactions: A role for JAMs in inflammatory cell recruitment

Several heterophilic interactions have been described for JAMs. Besides the aforementioned heterophilic interaction *in trans* between JAM-B and JAM-C (Arrate et al., 2001; Liang et al., 2002), several lines of evidence point to the fact that JAMs are also engaged as counter-receptors for leukocyte integrins: In particular, JAM-A binds the leukocyte integrin αLß2 (LFA-1) (Ostermann et al., 2002), JAM-B associates with α4ß1 (VLA-4) (Cunningham et al., 2002) and JAM-C interacts with ·Mß2 (Mac-1) (Santoso et al., 2002), thereby indicating a potential function of JAMs in leukocyte emigration (Fig. 1).

Leukocyte activation and adhesion to the endothelium and the subsequent transendothelial migration are pivotal steps in the recruitment of cells to the inflamed tissue. This highly coordinated multistep process requires tight regulation of adhesive events including the regulation of expression of adhesion receptors, as well as the modification of the affinity and avidity of these adhesion receptors. While leukocyte rolling depends on selectin-carbohydrate interactions, the process of adhesion is predominantly mediated by members of the ß2-integrin family, LFA-1 (αLß2, CD11a/CD18), Mac-1 (αMß2, CD11b/CD18), and p150,95 (αXß2, CD11c/CD18). Additionally, ß1-integrins, especially VLA-4 (α4ß1) interact with endothelial counter-receptors such as ICAM-1 or VCAM-1 (Carlos and Harlan, 1994; Springer, 1994; Stewart et al., 1995; Muller, 2002). In contrast to rolling and adhesion of leukocytes, the molecular mechanisms of leukocyte trans-endothelial migration are still not completely understood. Here, the recent findings on the JAM family members might provide important new pieces for the puzzle of leukocyte transendothelial migration (diapedesis).

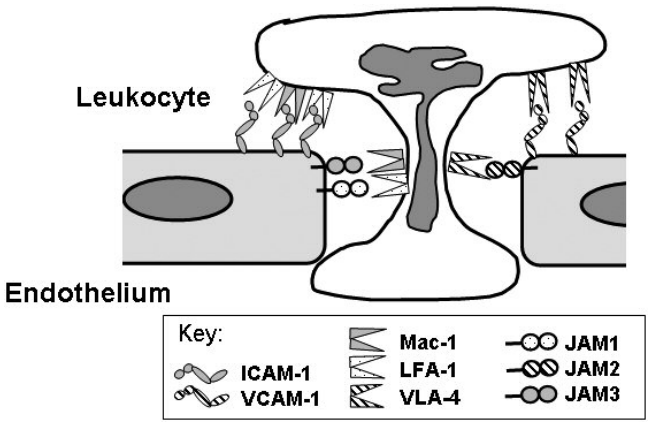

Fig. 1. After the fact that leukocytes firmly adhere to the endothelial cell surface (a process mediated by the interactions of ß2-integrins Mac-1 and LFA-1 with their endothelial counterreceptor ICAM-1 as well as the interaction of the ß1-integrin VLA-4 with its counterreceptor VCAM-1) leukocytes engage their ß2- and ß1-integrins, which interact with JAM-family members to pass the most apical regions of the interendothelial junctions. JAM-A is a counterreceptor of LFA-1, JAM-B is a counterreceptor of VLA-4 and JAM-C is a counterreceptor of Mac-1.

Diapedesis is a rapid process in which the inflammatory cell migrates between tightly opposed endothelial cells *in vitro* within minutes (Muller, 2003). Leukocyte transmigration might preferentially occur at tricellular corners, where the borders of three neighbouring endothelial cells intersect, as both adherens and tight junctions seem to have discontinuities at such sites (Burns et al., 1997). However, other experiments indicate that inflammatory cells preferentially migrate across junctions between two endothelial cells (Shaw et al., 2001).

Cellular localization, and homophilic as well as heterophilic binding capacities, pointed to a potential role of JAMs in leukocyte diapedesis. A first evidence for a putative role of JAM-A in diapedesis derived from experiments demonstrating inhibition of monocyte transendothelial migration *in vitro* and *in vivo* with antibodies against JAM-A (Martin-Padura et al., 1998; Del Maschio et al., 1999). As this antibody exclusively binds dimeric rather than monomeric JAM-A structures, it is the homophilic interaction between monocyte JAM-A and endothelial JAM-A that probably mediates transmigration in this scenario (Bazzoni et al., 2000a). In addition, JAM-A was also shown to bind to the leukocyte ß2-integrin LFA-1 and this heterophilic interaction of JAM-A is mediated by the membrane-proximal Ig domain of JAM-A thereby leaving the membrane-distal Ig domain available for homophilic dimerization at interendothelial junctions (Ostermann et al., 2002). The interaction between LFA-1 and JAM-A promotes transendothelial migration of lymphocytes already attached to the endothelium. Moreover, due to the inflammation-induced redistribution of JAM-A from junctions to the apical cell surface upon combined treatment with TNF-α and IFN-γ (Ozaki et al., 1999) the interaction between LFA-1 and JAM-A might also mediate leukocyte adhesion to the endothelium. In addition to leukocyte-endothelial interactions, JAM-A may also contribute to platelet-endothelial interactions (Naik et al., 2001), especially upon cytokine pre-stimulation of endothelial cells (Babinska et al., 2002). Finally, a further heterophilic interaction between JAM-A and αvß3 that occurs in cis manner on endothelial cells has been recently demonstrated. This JAM-integrin interaction regulates basic fibroblast growth factor-mediated ERK-activation and migration of endothelial cells (Naik et al., 2003a,b), and therefore, JAM-A may play an important role in basic fibroblast growth factor-induced angiogenesis.

JAM-B is expressed on vascular and lymphatic endothelium and especially in high endothelial venules and has therefore been proposed to play a special role in lymphocyte homing (Palmeri et al., 2000). Apart from JAM-C (Arrate et al., 2001; Liang et al., 2002), JAM-B also interacts with the leukocyte ß1-integrin VLA-4 (α4ß1) (Cunningham et al., 2002). It is noteworthy that this association is only efficient in case of an already existing JAM-B/JAM-C interaction (Cunningham et al., 2002). Whether the interaction between VLA-4 and JAM-B participates in leukocyte transendothelial migration remains to be investigated.

The third JAM-family member, JAM-C, was found to be a counter-receptor of the leukocyte ß2-integrin Mac-1 (Santoso et al., 2002). This heterophilic binding was found to mediate a firm platelet-leukocyte interaction especially under low-shear rate. Such an interaction between leukocytes and deposited platelets might be relevant for the recruitment of leukocytes at sites of vascular injury, where the endothelial cell lining has been denuded, e.g. in the atherosclerotic plaque. On the other hand, endothelial JAM-C also seems to promote neutrophil transmigration in a Mac-1-dependent manner (Chavakis et al., 2004, in press). In addition, JAM-C mediates neutrophil transepithelial migration in a Mac-1-dependent manner (Zen et al., 2004). Furthermore, the ectopic JAM-C overexpression in an endothelioma cell-line increased lymphocyte transmigration (Johnson-Leger et al., 2002). However, in contrast to neutrophils, lymphocytes do express JAM-C, thus, lymphocyte transendothelial migration might also be attributable to a homophilic interaction of JAM-C and not to its propensity to act as a counter-receptor for the ß2-integrin Mac-1. Taken together, it remains to be investigated under which (patho-)physiological conditions the homophilic or heterophilic interactions of JAM-C may be important. In this respect, the precise expression and localization of JAM-C on different vascular endothelial beds, as well as its regulation, needs to be determined.

Apart from JAMs, other molecules such as platelet-endothelial-cell adhesion molecule-1 (PECAM-1, CD31), CD99 and VE-cadherin, which are localized at the lateral borders of endothelial cells, have been implicated in leukocyte transmigration. Both PECAM-1 and CD99 are found on endothelial cells and most leukocytes and can undergo a homophilic interaction, which is crucial for transendothelial migration. Inhibition of PECAM-1 blocks leukocyte diapedesis *in vitro* and *in vivo* and leukocytes were found tightly attached to the apical surface without further passaging through the intercellular cleft (Vaporciyan et al., 1993; Bogen et al., 1994; Liao et al., 1995, 1997; Christofidou-Solomidou et al., 1997). In contrast, CD99 blockade reduced transmigration by arresting monocytes halfway across the endothelial junction, indicating that the CD99-dependent step lies distal to the PECAM-1-dependent step (Schenkel et al., 2002). Finally, VE-cadherin acts as a gatekeeper for the passage of leukocytes, since blockade of VE-cadherin increases the rate of neutrophil extravasation *in vivo* (Gotsch et al., 1997), whereas *in vitro* studies indicate that VE-cadherin is transiently removed from the junctions during diapedesis (Shaw et al., 2001). Whether the disappearance of VE cadherin from the junction at the site of leukocyte transmigration is a prerequisite for the process or a consequence of it, is yet unclear. Taken together, detailed studies need to be conducted in order to clarify the participation of JAMs and the rest pathways as well as the cooperation among

them in the regulation of leukocyte transendothelial migration.

Perspectives

Due to their localization at intercellular junctions, as well as on several circulating blood cells and their propensity to undergo a number of homophilic and heterophilic interactions, JAMs are implicated in different processes such as leukocyte transendothelial migration with relevance in the context of inflammation and the immune response, as well as in the formation and maintenance of tight junctions, thereby regulating paracellular permeability. Permeability changes are essential during angiogenesis and tumor biology and recent findings point to a functional role of JAM-A in endothelial cell biology and neovascularization (Naik et al., 2003a). Thus, future investigations on a potential role of JAMs in tumor growth, metastasis and angiogenesis need to be conducted. Moreover, as at present most data only exists for JAM-A, detailed studies on JAM-B and JAM-C need to be performed. In particular, it needs to be clarified which functions the three molecules share and which functions are distinct. In this respect, studies with mice deficient in one or more members of the JAM family will be instructive.

Note added in proof: Recently, JAM-C deficient mice displayed a defect in spermatogenesis. In particular, the interaction of JAM-C with PDZ-domain-containing molecules is essential for the polarization of round spermatids (Gliki et al., 2004).

References

Arrate M.P., Rodriguez J.M., Tran T.M, Brock T.A. and Cunningham S.A. (2001). Cloning of human junctional adhesion molecule 3 (JAM3) and its identification as the JAM2 counterreceptor. J. Biol. Chem. 276, 45826-45832.

Aurrand-Lions M.A., Duncan L., Ballestrem C. and Imhof B.A. (2001a). JAM-2, a novel immunoglobulin superfamily molecule, expressed by endothelial and lymphatic cells. J. Biol. Chem. 276, 2733-2741.

Aurrand-Lions M.A., Johnson-Leger C., Wong C., DuPasquier L. and Imhof B.A. (2001b). Heterogeneity of endothelial junctions is reflected by differential and specific subcellular localization of three JAM family members. Blood 98, 3699-3707.

Babinska A., Kedees M.H., Athar H., Ahmed T., Batuman O., Ehrlich Y.H., Hussain M.M. and Kornecki E. (2002). F11-receptor (F11R/JAM) mediates platelet adhesion to endothelial cells: role in inflammatory thrombosis. Thromb. Haemost. 88, 843-850.

Barclay A.N., Brown M.H., Law S.K.A., McKnight A.J., Tomlinson M.G. and van der Merwe P.A. (1997). The leukocyte antigen factsbook. Academic Press. London.

Bazzoni G. (2003). The JAM family of junctional adhesion molecules. Curr. Opin. Cell. Biol. 15, 525-530.

Bazzoni G., Martinez-Estrada O.M., Mueller F., Nelboeck P., Schmid G., Bartfai T., Dejana E. and Brockhaus M. (2000a). Homophilic interaction of junctional adhesion molecule. J. Biol. Chem. 275, 30970-30976.

Bazzoni G., Martinez-Estrada O.M., Orsenigo F.M., Cordenonsi M., Citi S. and Dejana E. (2000b). Interaction of junctional adhesion molecule with the tight junction components ZO-1, cingulin, and occludin. J. Biol. Chem. 275, 20520-20526.

Boettner B., Govek E.E., Cross J. and Van Aelst L. (2000). The junctional multidomain protein AF-6 is a binding partner of the Rap1A GTPase and associates with the actin cytoskeletal regulator profiling. Proc. Natl. Acad. Sci. USA 97, 9064-9069.

Bogen S., Pak J., Garifallou M., Deng X. and Muller W.A. (1994). Monoclonal antibody to murine PECAM-1 (CD31) blocks acute inflammation *in vivo*. J. Exp. Med. 179, 1059-1064.

Burns A.R., Walker D.C., Brown E.S., Thurman L.T., Bowden R.A., Keese C.R., Simon S.I., Entman M.L. and Smith V.C. (1997). Neutrophil transendothelial migration is independent of tight junctions and occurs preferentially at tricellular corners. J. Immunol. 159, 2893-2903

Carlos T.M. and Harlan J.M. (1994). Leukocyte-endothelial adhesion molecules. Blood 84, 2068-2071.

Chavakis T., Preissner K.T. and Santoso S. (2003). Leukocyte trans-endothelial migration: JAMs add new pieces to the puzzle. Thromb. Haemost. 89, 13-17.

Chavakis T., Keiper T., Matz-Westphal R., Hersemeyer K., Sachs U.J., Nawroth P.P., Preissner K.T. and Santoso S. (2004). The juntional adhesion molecule-C promotes neutrophil transendothelial migration *in vitro* and *in vivo*. J. Biol. Chem. (in press).

Christofidou-Solomidou M., Nakada M.T., Williams J., Muller W.A. and DeLisser H.M. (1997). Neutrophil platelet endothelial cell adhesion molecule-1 participates in neutrophil recruitment at inflammatory sites and is down-regulated after leukocyte extravasation. J. Immunol. 158, 4872-4878.

Cunningham S.A., Arrate M.P., Rodriguez J.M., Bjercke R., Vanderslice P., Morris A.P. and Brock T.A. (2000). A novel protein with homology to the junctional adhesion molecule: characterization of leukocyte interactions. J. Biol. Chem. 275, 43750-4356.

Cunningham S.A., Rodriguez J.M., Arrate P.M., Tran T.M. and Brock T.A. (2002). JAM2 interacts with alpha4beta1. Facilitation by JAM3. J. Biol. Chem. 277, 27589-27592.

Dejana E., Corada M. and Lampugnani M. (1995). Endothelial cell-to-cell junctions. FASEB J. 9, 910-918.

Dejana E., Spagnuolo R. and Bazzoni G. (2001). Interendothelial junctions and their role in the control of angiogenesis, vascular permeability and leukocyte transmigration. Thromb. Haemost. 86, 308-315.

DelMaschio A., de Luigi A., Martin-Padura I., Brockhaus M., Bartfai T., Fruscella P., Adorini L., Martino G., Furlan R., de Simoni M.G. and Dejana E. (1999). Leukocyte recruitment in the cerebrospinal fluid of mice with experimental meningitis is inhibited by an antibody to junctional adhesion molecule (JAM). J. Exp. Med. 190, 1351-1356.

Ebnet K., Schulz C.U., Meyer Zu Brickwedde M.K., Pendl G.G. and Vestweber D. (2000). Junctional adhesion molecule interacts with the PDZ domain-containing proteins AF-6 and ZO-1. J. Biol. Chem. 275, 27979-27988.

Ebnet K., Suzuki A., Horikoshi Y., Hirose T., Meyer Zu Brickwedde M.K., Ohno S. and Vestweber D. (2001). The cell polarity protein ASIP/PAR-3 directly associates with junctional adhesion molecule (JAM). EMBO J. 20, 3738-3748.

Ebnet K., Aurrand-Lions M., Kuhn A., Kiefer F., Butz S., Zander K., Meyer Zu Brickwedde M.K., Suzuki A., Imhof B.A. and Vestweber D. (2003). The junctional adhesion molecule (JAM) family members

JAM-2 and JAM-3 associate with the cell polarity protein PAR-3 : A possible role for JAMs in endothelial cell polarity. J. Cell. Sci. 116, 3879-3791.

Ebnet K., Suzuki A., Ohno A. and Vestweber D. (2004). Junctional Adhesion Molecules (JAMs): more molecules with dual functions?. J. Cell. Sci. 117, 19-29.

Fanning A.S., Jameson B.J., Jesaitis L.A. and Anderson J.M. (1998). The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. J. Biol. Chem. 273, 29745-29753.

Gao L., Joberty G. and Macara I.G. (2002) Assembly of epithelial tight junctions is negatively regulated by Par6. Curr. Biol. 12, 221-225.

Gilik G., Ebnet K., Aurrand-Lions M., Imhof B.A. and Adams R.H. (2004). Spermatid differentiation requires the assembly of a cell polarity complex downstream of junctional-adhesion-molecule-C. Nature 431, 320-324.

Gotsch U., Borges E., Bosse R., Boggemeyer E., Simon M., Mossmann H. and Vestweber D. (1997). VE-cadherin antibody accelerates neutrophil recruitment *in vivo*. J. Cell. Sci. 110, 583-588.

Hamazaki Y., Itoh M., Sasaki H., Furuse M. and Tsukita S. (2002). Multi-PDZ domain protein 1 (MUPP1) is concentrated at tight junctions through its possible interaction with claudin-1 and junctional adhesion molecule. J. Biol. Chem. 277, 455-461.

Haskins J., Gu L., Wittchen E.S., Hibbard J. and Stevenson B.R. (1998). ZO-3, a novel member of the MAGUK protein family bound at the tight junction, interacts with ZO-1 and occludin. J. Cell Biol. 141, 199-208.

Hirabayashi S., Tajima M., Yao I., Nishimura W., Mori H. and Hata Y. (2003). JAM4, a junctional cell adhesion molecule interacting with the tight junction protein MAGI-1. Mol. Cell. Biol. 23, 4267-4282.

Itoh M., Furuse M., Morita K., Kubota K., Saitou M. and Tsukita S. (1999). Direct binding of three junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH-termini of claudins. J. Cell Biol. 147, 1351-1363.

Joberty G., Petersen C., Gao L. and Marcara I.G. (2000). The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. Nat. Cell. Biol. 2, 531-539.

Johansson A., Driessens M. and Aspenstrom P. (2000). The mammalian homologue of the caenorhabditis elegans polarity protein PAR-6 is a binding partner for the Rho GTPases cdc42 and rac1. J. Cell. Sci. 13, 3267-3275.

Johnson-Leger C., Aurrand-Lions M. and Imhof B.A. (2000). The parting of the endothelium: miracle, or simply a junctional affair? J. Cell. Sci. 113, 921-933.

Johnson-Leger C.A., Aurrand-Lions M., Beltraminelli N., Fasel N. and Imhof B.A. (2002). Junctional adhesion molecule–2 (JAM-2) promotes lymphocyte transendothelial migration. Blood 100, 2479-2486.

Kemler R. (1993). From cadherins to catenins : cytoplasmic protein interactions and regulation of cell adhesion. Trends. Genet. 9, 317-321.

Kornecki E., Walkowiak B., Naik U.P. and Ehrlich Y.H. (1990). Activation of human platelets by a stimulatory monoclonal antibody. J. Biol. Chem. 265, 10042-10048.

Kostrewa D., Brockhaus M., D'Arcy A., Dale G.E., Nelboeck P., Schmid G., Mueller F., Bazzoni G., Dejana E., Bartfai T., Winkler F.K. and Hennig M. (2001). X-ray structure of junctional adhesion molecule : structural basis for homophilic adhesion via a novel dimerization motif. EMBO J. 20, 4391-4398.

Kuriyama M., Harada N., Kuroda S., Yamamoto T., Nakafuku M., Iwamatsu Y., Yamamoto D., Prasad R., Croce C., Canaani E. and Kaibuchi K. (1996). Identification of AF-6 and canoe as a putative targets for Ras. J. Biol. Chem. 271, 607-610.

Liang T.W., DeMarco R.A., Mrsny R.J., Gurney A., Gray A., Hooley J., Aaron H.L., Huang A., Klassen T., Tumas D.B. and Fong S. (2000). Characterization of huJAM : evidence for involvement in cell-cell contact and tight junction regulation. Am. J. Physiol. Cell. Physiol. 279, c1733-c1734.

Liang T.W., Chiu H.H., Gurney A., Sidle A., Tumas D.B., Schow P., Foster J., Klassen T., Dennis K., DeMarco R.A., Pham T., Frantz G. and Fong S. (2002). Vascular endothelial-junctional adhesion molecule (VE-JAM)/JAM 2 interacts with T, NK, and dendritic cells through JAM 3. J. Immunol. 168, 1618-1626.

Liao F., Huynh H.K., Eiroa A., Greene T., Polizzi E. and Muller W.A. (1995). Migration of monocytes across endothelium and passage through extracellular matrix involve separate molecular domains of PECAM-1. J. Exp. Med. 182, 1337-1343.

Liao F., Ali J., Greene T. and Muller W.A. (1997). Soluble domain 1 of platelet endothelial cell adhesion molecule (PECAM) is sufficient to block transendothelial migration *in vitro* and *in vivo*. J. Exp. Med. 185, 1349-1357.

Lin D., Edwards A.S., Fawcett J.P., Mbamalu G., Scott J.D. and Pawson T. (2000) A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. Nat. Cell. Biol. 2, 540-547.

Liu Y., Nusart A., Schnell T.J., Reaves Tam Walsh S., Pochet M. and Parkos C.A. (2000). Human junction adhesion molecule regulates tight junction resealing in epithelia. J. Cell. Sci. 113, 2363-2374.

Martin-Padura I., Lostaglio S., Schneemann M., Williams L., Romano M., Fruscella P., Panzeri C., Stoppacciaro A., Ruco L., Villa A., Simmons D. and Dejana E. (1998). Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and moderated monocyte transmigration. J. Cell. Biol. 142, 117-127.

Meyer T.N., Schwesinger C. and Denker B.M. (2002). Zonula occludens-1 is a scaffolding protein for signaling molecules. Galpha (12) directly binds to the Src homology 3 domain and regulates paracellular permeability in epithelial cells. J. Biol. Chem. 277, 24855-24858.

Meyer T.N., Hunt J., Schwesinger C. and Denker B.M. (2003). Galpha12 regulates epithelial cell junctions through Src tyrosine kinases. Am. J. Physiol. Cell. Physiol. 285, C1281-C1293.

Muller W.A. (2002). Leukocyte-endothelial cell interactions in the inflammatory response. Lab. Invest. 82, 521-533.

Muller W.A. (2003). Leukocyte-endothelial-cell interactions in leukocyte transmigration and the inflammatory response. Trends Immunol. 24, 326-332.

Naik U.P., Ehrlich Y.H. and Kornecki E. (1995). Mechanisms of platelet activation by a stimulatory antibody : cross-linking of a novel platelet receptor for monoclonal antibody F11 with the Fc gamma RII receptor. Biochem. J. 310, 155-162.

Naik U.P., Naik U.M., Eckfeld K., Martin-DeLeon P. and Spychala J. (2001). Characterization and chromosomal localization of JAM-1, a platelet receptor for a stimulatory monoclonal antibody. J. Cell. Sci. 114, 539-547.

Naik M.U., Mousa S.A., Parkos C.A. and Naik U.P. (2003a). Signaling through JAM-1 and {alpha}v{beta}3 is required for the angiogenic action of bFGF : dissociation of JAM-1 and {alpha}v{beta}3 complex.

Blood 102, 2108-2114.

Naik M.U., Vuppalanchi D. and Naik UP. (2003b). Essential role of junctional adhesion molecule-1 in basic fibroblast growth-factor induced endothelial cell migration. Arterioscler. Thromb. Vasc. Biol. 23, 2165-2171.

Ostermann G., Weber K.S., Zernecke A., Schroeder A. and Weber C. (2002). JAM-1 is a ligand of the beta(2) integrin LFA-1 involved in transendothelial migration of leukocytes. Nat. Immunol. 3, 151-158.

Ozaki H., Ishii K., Horinchi H., Arai H., Kawamoto T., Okawa K., Iwamatsu A. and Kita T. (1999). Cutting edge: combined treatment of TNF-alpha and IFN-gamma causes redistribution of junctional adhesion molecules in human endothelial cells. J. Immunol. 163, 553-557.

Palmeri D., van Zante A., Huang C.C., Hemmerich S. and Rosen S.D. (2000). Vascular endothelial junction-associated molecule, a novel member of the immunoglobulin superfamily, is localized to intercellular boundaries of endothelial cells. J. Biol. Chem. 275, 19319-19345.

Qiu R.G., Abo A. and Martin G.S. (2000) A human homologue of the C. elegans polarity determinant PAR-6 links Rac and Cdc42 to PKCzeta signalling and cell transformation. Curr. Biol. 10, 697-707.

Rajasekaran A.K., Hojo M., Huima T. and Rodriguez-Boulan E. (1996). Catenins and zonula occludens-1 form a complex during early stages in the assembly of tight junctions. J. Cell Biol. 132, 451-463.

Saha C., Nigam S.K. and Denker B.M. (2001). Expanding role of G proteins in tight junction regulation: Galpha (s) stimulates TJ assembly. Biochem. Biophys. Res. Commun. 285, 250-256.

Santoso S., Sachs U.J.H., Kroll H., Linder M., Ruf A., Preissner K.T. and Chavakis T. (2002). The junctional adhesion molecule 3 (JAM-3) on human platelets is a counterreceptor for the leukocyte integrin Mac-1. J. Exp. Med. 196, 679-691.

Schenkel A.R., Mamdouh Z., Chen X., Liebman R.M. and Miller W.A. (2002). CD99 plays a major role in the migration of monocytes through endothelial junctions. Nat. Immunol. 3, 143-150.

Shaw S.K., Bamba P.S., Perkins B.N. and Luscinskas F.W. (2001). Real-time imaging of vascular endothelial-cadherin during leukocyte transmigration across endothelium. J. Immunol. 167, 2323-2330.

Sobocka M.B., Sobocki T., Banerjee P., Weiss C., Rushbrook J.I., Norin A.J., Hartwig J., Salifu M., Markell M.S., Babinska A., Ehrlich Y.H. and Kornecki E. (2000). Cloning of the human platelet F11-receptor: a cell adhesion molecule member of the immunoglobulin superfamily involved in platelet aggregation. Blood 95, 2600-2609.

Springer T.A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration : a multistep paradigm. Cell 76, 301-314.

Stewart M., Thiel M. and Hogg, N. (1995). Leukocyte integrins. Curr. Opin. Biol. 7, 690-696.

Suzuki A., Yamanaka T., Hirose T., Manabe N., Mizuko K., Shimizu M., Akimoto K., Izumi Y., Ohnishi T. and Ohno S. (2001). Atypical protein kinase C is involved in the evolutionary conserved PAR protein complex and plays a critical role in establishing epithelia-specific junctional structures. J. Cell Biol. 152, 1183-1196.

Vaporciyan A.A., DeLisser H.M., Yan H.C., Mendiguren I.I., Thom S.R., Jones M.L., Ward P.A. and Albelda S.M. (1993). Involvement of platelet-endothelial cell adhesion molecule-1 in neutrophil recruitment *in vivo*. Science 262, 1580-1582.

Williams A.F and Barclay A.N. (1988). The immunoglobulin superfamily – domains for cell surface recognition. Annu. Rev. Immunol. 6, 381-405.

Wittchen E.S., Haskins J. and Stevenson B.R. (1999). Protein interactions at the tight junction. Actin has multiple binding partners, and ZO-1 forms independent complexes with ZO-2 and ZO-3. J. Biol. Chem. 274, 35179-35185.

Yamamoto T., Harada N., Kano K., Taya S., Canaani E., Matsuura Y., Mizoguchi A., Ide C. and Kaibuchi K. (1997). The Ras target AF-6 interacts with ZO-1 and serves as a peripheral component of tight junctions in epithelial cells. J. Cell Biol. 139, 785-795.

Zen K., Babbin B.A., Liu Y., Whelan J.B., Nusrat A. and Parkos C.A. (2004). JAM-C is a component of desmosomes and a ligand for CD11b/CD18- mediated neutrophil transepithelial migration. Mol. Cell. Biol. 15, 3926-3937.

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