Vascular and epithelial junctions: a barrier for leucocyte migration

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Abstract
Rapid mobilization of leucocytes through endothelial and epithelial barriers is key in immune system reactivity. The underlying mechanisms that regulate these processes have been the basis for many recent studies. Traditionally, leucocyte extravasation had been believed to occur through a paracellular route, which involves localized disruption of endothelial cell junctions. However, more recently, a transcellular route has been described involving the passage through the endothelial cell body. Leucocytes are also able to migrate through epithelium to monitor mucosal tissues and microenvironments. A number of adhesion molecules are known to regulate transmigration of leucocytes through epithelial and endothelial layers. Paracellular and transcellular leucocyte transmigration are regulated by adhesion molecules such as PECAM-1 (platelet-endothelial cell adhesion molecule 1), CD99, VE-cadherin (vascular endothelial cadherin) and JAM (junctional adhesion molecule) proteins. The purpose of this review is to discuss the role of these molecules in leucocyte transmigration and how they contribute to the different mechanisms that regulate leucocyte trafficking.

Introduction
The proficiency of the innate and adaptive immune response requires efficient circulation of leucocytes between blood, lymphoid organs and peripheral tissue compartments. Critically, leucocyte circulation involves passage through the endothelial barrier without affecting vessel integrity. Several complementary techniques have been employed to analyse the molecular mechanisms of leucocyte transmigration. Studies using blocking antibodies and gene-deficient animals have made a significant contribution to our understanding of the roles of different molecules that regulate leucocyte transmigration. More recently, time-lapse and intravital microscopy studies have allowed a more in-depth understanding of the dynamics of leucocyte trafficking. In the present review, we will highlight the molecular mechanisms that are involved in leucocyte transmigration through vascular and epithelial tissues.

Transendothelial migration
The mechanism of leucocyte migration across the endothelium is a complex multistep process, which includes leucocyte tethering, rolling, tight adhesion and extravasation [1]. Selectins, expressed by activated endothelial cells and leucocytes, bind to ligands on opposing cells (Figure 1). These interactions mediate leucocyte capture from flow and tethering to endothelium. The high dissociation/association rates of selectins allow leucocytes to roll in the direction of flow and sense activation signals on the endothelial wall. Chemokines presented on luminal surfaces trigger rapid activation of leucocyte integrins, leading to rolling arrest and firm adhesion. Secondary adhesion events are mediated by leucocyte integrins [LFA-1 (leucocyte function-associated antigen 1), VLA-4 (very late antigen 4) and Mac-1], which bind to endothelial adhesion molecules such as ICAM-1 (intercellular adhesion molecule 1) and VCAM-1 (vascular cell adhesion molecule 1) (Figure 1). The final step of this cascade is extravasation or diapedesis, a process where leucocytes must migrate either between two or three adjacent endothelial cells or across the endothelial cell body [2] (Figures 1 and 2). Adhesion molecules such as PECAM-1 (platelet–endothelial cell adhesion molecule 1), CD99 and JAMs (junctional adhesion molecules) are known to mediate these events [3]. In addition, it has been shown that adherent leucocytes can induce an increase in intracytoplasmic calcium levels in endothelial cells. This in turn activates MLCK (myosin light-chain kinase), resulting in myosin skeleton reorganization and retraction of the cell body [4]. This phenomenon takes place only in endothelial cells adjacent to transmigrating leucocytes and facilitates leucocyte migration across the endothelial monolayer [5]. Transmigration is controlled further by soluble factors secreted by activated leucocytes such as CAP37/HBP, a heparin-binding protein [6].

Transcellular or paracellular route?
Classically, leucocyte transmigration was viewed as leucocyte migration between adjacent endothelial cells. However, studies using electron microscopy have provided evidence...
that leucocytes can also migrate directly through the endothelial cell body [2,7]. In both types of migration, microvillus-like projections enriched in ICAM-1 have been observed around migrating leucocytes, suggesting that both pathways may have a similar dependence on ICAM-1. A recent study has shown that the cytoplasmic tail of ICAM-1, together with higher expression levels, is required for high transcellular transmigration [8]. In fact, the truncation of the cytoplasmic tail of ICAM-1 was shown to direct leucocytes to a paracellular transmigration route. This difference in ICAM-1-dependency may be due to the presence of alternative adhesion molecules in endothelial junctions highlighted by a number of recent studies. An endothelial ‘cup-like’ structure, called a ‘podoprint’, has been shown to form around migrating leucocytes during initial stages of transcellular migration of lymphocytes [9]. These podoprints are composed of ICAM-1 and VCAM-1 in a caveolin-rich structure, possibly linked to the cytoskeleton protein vimentin [10,11]. Mechanistically, transcellular migration is driven by internalization of ICAM-1 by the caveolin-rich domains forming channels through which lymphocytes cross the endothelial cell body [12] (Figure 2). Surprisingly, activated lymphocytes also use PECAM-1 for transcellular migration, whereas non-activated lymphocytes ignore PECAM-1 during transmigration [9,13]. A cup-like structure also forms around activated lymphocytes, but contains PECAM-1 in addition to ICAM-1. Thus, although mechanisms used for both routes of transmigration seem to differ, common adhesion molecules may be involved. The factors which influence the choice between the two migrating routes, however, remain to be found.

It was observed recently that approx. 30% of IL (interleukin)-2-activated T-lymphocytes, a small percentage of the leucocyte population, take the transcellular route through microvascular cells compared with only 10% through macrovascular cells [9]. Interestingly, a recent study using a HUVEC (human umbilical vein endothelial cell) model suggests that naïve lymphocytes utilize the transcellular route in macrovascular cells [11]. Taken together, these data suggest that the transcellular route, under certain conditions, is not a marginal phenomenon and may play a major role under certain conditions. Transcellular migration may be particularly relevant in organs that have extremely tight endothelial junctions, such as the brain. In this case, movement through the endothelial cell body may represent the safest and least resistant pathway across the vascular barrier into the surrounding tissue [14].
**Figure 2 | Transcellular endothelial migration**

In transcellular migration, leucocytes do not migrate to the junctional region and remain at the apical surface of the endothelial cell. Formation of an ICAM-1-LFA-1-enriched invagination allows leucocytes to penetrate the endothelial cell. After crossing the cell body, leucocytes are released into the tissue. BM, basement membrane.

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**The mechanism of paracellular transmigration**

Paracellular transmigration allows leucocyte passage across the endothelium without affecting endothelial integrity. Indeed, several studies (e.g., [15]) have shown that leucocytes can cross the endothelial barrier without increasing permeability. Thus adhesion molecules at the endothelial junction have a dual function in maintaining endothelial cell–cell contacts and regulating leucocyte transmigration. In order to cross the endothelium, leucocytes must breach the tight junctional contacts with a continuous disruption and closing of the junctions. Attention has been given to adhesion molecules that are involved in leucocyte transmigration, such as PECAM-1, VE-cadherin (vascular endothelial cadherin), CD99 and JAMs. The function of each molecule in transendothelial migration is discussed below.

**PECAM-1**

PECAM-1 is a member of the IgSF (immunoglobulin superfamily) and is expressed on endothelial cells, leucocytes and platelets. In non-activated endothelial cells, PECAM-1 is concentrated at intercellular junctions where it can mediate homophilic interactions [16]. Several studies *in vitro* and *in vivo* have shown that PECAM-1 blockade can inhibit the transmigration of neutrophils and monocytes, but not lymphocytes, without affecting adhesion on to the endothelial surface [17,18]. PECAM-1 has been shown to be involved in regulating two distinct stages of leucocyte transmigration (Figure 1). Anti-PECAM-1 antibodies directed against the first two Ig domains and a soluble molecule containing the first PECAM-1 Ig domain reduced transmigrating leucocytes at the junctional barrier, resulting in accumulation of leucocytes on the luminal surface [19,20]. The second distinct step in which PECAM-1 is involved has been shown to occur later in the transmigration cascade. In fact, an antibody directed against the last PECAM-1 Ig domain induced accumulation of leucocytes at the endothelial–basal membrane interface [18,21]. However, leucocyte recruitment in inflammatory pathology models is only delayed in PECAM-1-deficient mice, as summarized in a recent review [22]. This suggests that other adhesion molecules compensate for PECAM-1 deficiency *in vivo*. Indeed, Huang et al. [23] showed that ICAM-2 is involved in PECAM-1-independent leucocyte migration. High expression of ICAM-2 has been observed at endothelial cell contacts, and interactions on leucocytes with LFA-1 and Mac-1 (CD11b/CD18) have been described. Furthermore, ICAM-2-blocking antibody inhibited neutrophil transmigration at the same level in wild-type and PECAM-1-deficient mice. In summary, PECAM-1 is a protein that is involved in, but not essential for, transendothelial leucocyte migration.

**CD99**

CD99 is an O-glycosylated protein expressed by most leucocytes, erythrocytes and endothelial cells. Shown to be a T-cell co-stimulatory molecule [24], CD99 also plays a role...
in monocyte transmigration [25]. A subsequent in vivo study demonstrated that anti-CD99 antibody inhibited transmigration of activated lymphocytes into inflammatory sites, but did not affect homing of naïve lymphocytes [26]. Similarly to PECAM-1, CD99 can mediate homophilic interaction between endothelial cells and migrating leucocytes [25]. Blocking both CD99 and PECAM-1 had an additive effect in reducing monocyte transmigration [25]. Analysis by confocal microscopy has shown that CD99 acts after the first PECAM-1-involved step during transmigration. Pre-treatment with blocking antibodies led to monocytes being trapped within the lateral membranes of the endothelial cells [25].

**VE-cadherin**

VE-cadherin is specifically expressed at adherens junctions of endothelium and is important for endothelial cell-contact stability. VE-cadherin interacts homophilically in a calcium-dependent manner [27]. It is linked to the actin cytoskeleton by catenin complexes. During leucocyte transmigration, VE-cadherin and the associated catenins transiently disappear from the endothelial cell membrane through which leucocytes are migrating, but junctional complexes are reconstituted rapidly after transmigration [28] (Figure 1). In vivo, anti-VE-cadherin antibody increased neutrophil migration into sites of inflammation [29]. Blocking VE-cadherin function at cell junctions may facilitate leucocyte transmigration by increasing the turnover of this molecule. Physiologically, reduction of VE-cadherin at cell–cell junctions could be due to phosphorylation of the molecule by Src and/or Pyk2 tyrosine kinases. It has been shown that ICAM-1 engagement activates these two kinases, leading to VE-cadherin phosphorylation, required for efficient transmigration of neutrophils [30]. Moreover, knockdown of VE-PTP (vascular endothelial protein tyrosine phosphatase) led to increased neutrophil transmigration [3]. Overall, these studies have demonstrated a clear role for VE-cadherin in leucocyte transmigration, and that phosphorylation of its cytoplasmic tail plays an integral role in regulating this function.

**JAMs**

JAMs are V-C2 IgSF-type proteins and members of the CTX (cortical thymocyte marker of Xenopus) protein family. The JAM family is composed of three classical members, JAM-A, JAM-B and JAM-C, and four non-classical members, ESAM (endothelial-cell-specific adhesion molecule), CAR (coxsackievirus and adenovirus receptor), JAM4 and JAML, which have a longer cytoplasmic tail [31]. JAMs have proven to be of particular interest, as many members of this family have been implicated in regulating leucocyte transmigration [32–34]. JAM-A is expressed by epithelial and endothelial cells and megakaryocytes, and, in humans, by leucocytes and platelets [35]. Reports on the role of JAM-A in leucocyte transmigration are controversial. In several inflammatory models in mice, such as chemokine-loaded air-pouches or cytokine-induced meningitis, an antibody against murine JAM-A reduced leucocyte infiltration and inflammation [32,33]. In other models, such as meningitis induced by *Listeria monocytogenes* or lymphohcyt choriomeningitis virus, the same antibody failed to reduce leucocyte recruitment [36]. Two other studies involving human JAM-A have also led to contradictory results, one showing no effect of JAM-A blockade on leucocyte migration through microvascular cells [37], whereas the other describes a role for JAM-A in transmigration of T-cells and PMN (polymorphonuclear) leucocytes [38]. In the latter study, endothelial cells were stimulated with pro-inflammatory factors such as TNFα (tumour necrosis factor α) and IFNγ (interferon γ), which led to redistribution of JAM-A on to the apical surface [39]. This redistribution allowed interaction of JAM-A with the leucocyte integrin LFA-1 [38]. JAM-A is also expressed by DCs (dendritic cells) and it has been shown to negatively control DC trafficking to lymph nodes. In JAM-A-deficient mice, DCs migrating to lymph nodes, after FITC skin painting or induction of contact hypersensitivity, is increased [40]. Recently, Matsutani et al. [41] have shown that pDCs (plasmacytoid DCs) use JAM-A as a transmigration mediator on HEVs (high endothelial venules) in lymph nodes. An anti-JAM-A antibody reduced transmigration of pDCs, but did not affect adhesion to the endothelium, which is mediated by ICAM-1 and ICAM-2. Using an inflammatory peritonitis and cardiac ischaemia model, Corada et al. [42] have shown that JAM-A is essential for migration of PMN leucocytes through the endothelium. This is independent of JAM-A expression on endothelial cells, but depends on the presence of JAM-A on leucocytes [42]. Intravital studies using anti-JAM-A monoclonal antibody on wild-type mice or JAM-A-deficient mice showed that leucocyte transmigration through stimulated cremasteric venules was reduced when JAM-A function is disrupted [43]. Surprisingly, this was obtained when inflammation was induced by IL-1β, but not in response to PAF (platelet-activating factor) or LTβ4 (lymphotoxin β4). By conducting adoptive leucocyte transfer into JAM-A-deficient mice, the authors showed that leucocyte transmigration is dependent on vascular JAM-A. A dual-blockade study with PECAM-1 revealed that JAM-A and PECAM-1 act sequentially in leucocyte transmigration through the endothelial wall [43]. In conclusion, these findings demonstrate that the participation of JAM-A in leucocyte transmigration is variable and highly dependent on the nature of the inflammatory stimuli.

JAM-B is highly expressed by HEVs at intercellular junctions [44]. It interacts homophilically, but also mediates heterophilic interactions with JAM-C [45]. It has also been demonstrated that JAM-B can interact with the integrin VLA-4, expressed by lymphocytes, monocytes and eosinophils [46]. This interaction has been shown to be more efficient when JAM-B is already engaged with JAM-C [47]. Using commercial polyclonal antibodies, Ludwig et al. [48] showed that JAM-B and JAM-C contribute to leucocyte accumulation in DNFB (1-fluoro-2,4-dinitrobenzene)-induced dermatitis [48]. Antibody blockade of both JAM-B and JAM-C reduced recruitment of neutrophils to the site of inflammation in a dose-dependent manner. Furthermore, it
Reverse transendothelial migration is promoted by different factors such as ICAM-1 for leucocyte adhesion. It is also negatively controlled by JAM-C-JAM-B interaction, which may function as a retention mechanism. BM, basement membrane.

Figure 3 | Reverse transendothelial migration

was shown that JAM-B and JAM-C may have distinct roles in this process, as the effect of combining antibodies was additive. To date, no further studies on the role of JAM-B in leucocyte transmigration have been published, and a specific function for JAM-B in the inflammatory process remains to be elucidated.

JAM-C is expressed by most endothelial cells, fibroblasts, smooth muscle cells and epithelial cells. In humans, it is also expressed by a subpopulation of B-cells, T-cells, natural killer cells and DCs, and by platelets [35]. It has now been confirmed that expression seen previously on monocytes was due to adherent platelets [34,49]. One of the first studies to describe a role for JAM-C in lymphocyte transmigration was conducted using blocking antibodies and a soluble molecule, which blocked migration across HUVEC monolayers under static conditions in response to the chemokine SDF-1 (stromal-cell-derived factor 1) [34]. It is possible that JAM-C participates in this process by homophilic interactions similarly to PECAM-1 and CD99. However, heterophilic interactions with leucocyte integrin Mac-1 or endothelial JAM-B must also be considered [47,50]. Notably, interaction of Mac-1 with JAM-C has been described to be involved in neutrophil transmigration in static assays under similar conditions [51]. The contribution of JAM-C to leucocyte transmigration is independent of PECAM-1 and ICAM-1, as blocking with a soluble form of JAM-C combined with anti-PECAM-1 or anti-ICAM-1 was additive [51]. This suggests sequential action of the three molecules during transendothelial migration. In vivo, overexpression of endothelial JAM-C in transgenic mice enhanced neutrophil accumulation at the sites of inflammation [52]. In this study, intravital videomicroscopy revealed a role for endothelial JAM-C in adhesion and transmigration of neutrophils, but not in rolling. Co-culture experiments under flow have shown that JAM-C blockade reduced the number of monocytes in the basolateral compartment of the endothelial monolayer. Analysing transmigration of individual monocytes revealed that JAM-C blockade increased reverse transmigration of monocytes [49]. This was confirmed in vivo with increased numbers of reverse-transmigrated monocytes in the circulation after pretreatment with anti-JAM-C antibody. Thus JAM-C creates a vascular barrier facilitating one-way traffic of leucocytes through vascular endothelium towards inflammatory tissue.

Reverse transmigration of leucocytes is a process that has been described previously (Figure 3) [53]. The authors discovered this phenomenon by observing monocyte retention under a HUVEC monolayer cultured on an amniotic membrane [53]. Although transmigration of leucocytes was rapid, bulk reverse transmigration took several hours. In addition to JAM-C, reverse transmigration is controlled by ICAM-1, which was found on both luminal and basolateral surfaces of HUVECs [53]. The process of leucocyte reverse transmigration is thought to be a mechanism of tissue clearance contributing to the resolution of inflammation.
**Figure 4** | Transepithelial migration

Leucocytes adhere to the basolateral side of the epithelium by Mac-1–proteoglycan interaction, before transepithelial migration along the basolateral membrane. Adhesion molecules such as JAMs and CD47 promote this migration. Leucocytes reach the apical side of the epithelium and are retained at the surface of epithelial cells by ICAM-1–Mac-1 and Ig–FcR (Fc receptor) interaction. This allows neutrophils to get access to pathogens and to mediate an effective immune response. AJ, adherens junction; BM, basement membrane; TJ, tight junction.

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**JAM-related proteins**

As stated, ESAM, CAR, JAM4 and JAML are adhesion molecules belonging to a separate subgroup of the JAM family. They differ from the classical members by their longer intracellular tail, which contains a type-I instead of type-II PDZ domain [35]. Consequently, they bind to different cytoplasmic polarity complex components. For example, whereas JAMs bind to polarity complex molecules, such as the PARs (partition-defective proteins), through the type-I PDZ domain, ESAM and JAM4 interact with MAGI-1 (membrane-associated guanylate kinase protein 1) [54,55].

Expression of ESAM is restricted to endothelial cell tight junctions and to the surface of activated platelets [56]. After cytokine stimulation, ESAM-deficient mice showed delayed recruitment of neutrophils to the peritoneum. This effect was shown to be mediated by endothelial ESAM rather than by platelet ESAM [57]. One possibility is that ESAM deficiency contributes to neutrophil transmigration by impairing vascular permeability. This is consistent with ESAM-deficient mice showing reduced vascular permeability after induction by VEGF (vascular endothelial growth factor).

CAR has been shown to mediate homophilic interactions at cell–cell contacts as well as heterophilic interactions with JAM-C and JAML [58,59]. Expression of JAM-C on spermatogenic cells has been shown to play a critical role in the maturation of spermatids through an interaction with an unknown ligand on Sertoli cells [60]. It is therefore intriguing that a recent report described CAR–JAM-C interactions in the acrosomal region of spermatozoa [59]. This suggests that CAR could be the JAM-C ligand at the Sertoli–spermatogenic cell contacts. To date, no studies have shown a role of CAR in leucocyte transendothelial migration. Speculation based on the multiple ligand partners of CAR has recently become an active area of investigation.

JAM4 has been described on epithelial cells, male germ progenitor cells and haemopoietic cell lineages [61]. It has been shown to regulate epithelial monolayer permeability through its association with cytoplasmic MAGI-1 [54]. No heterophilic interactions have been described and there is no information on any role of JAM4 in leucocyte transmigration.

**Transepithelial migration**

Similarly to leucocyte reverse transendothelial migration, movement of leucocytes through the epithelial barrier is oriented from the basolateral to the apical side (Figure 4). This process is essential for innate immune defence in mucosal
Concluding remarks

In this review, we have highlighted the different adhesion molecules regulating leucocyte migration across endothelium and epithelium. The multiplicity of molecules implicated in leucocyte transmigration demonstrates that these processes are complex. Different leucocytes use different molecules to transmigrate and the relative importance of each adhesion molecule depends greatly on the type of vessels, leucocytes and stimuli used. This probably reflects a redundancy in the molecules implicated in each step of this process. Leucocyte migration through the endothelium is described as a process subdivided into tethering, rolling, adhesion, diapedesis and migration across the basal membrane. Each step is controlled by a set of different adhesion molecules, which function in a serial cascade to recruit leucocyte populations from the vascular lumen into sites of inflammation. However, we cannot exclude that unidentified molecules may control an as yet undescribed step. Although a lot of progress in the knowledge of leucocyte transmigration has been made throughout recent years, some points remain unclear and require further elucidation.

The physiological relevance of transcellular migration compared with paracellular migration is intriguing, but remains to be defined. Studies that have shown a role for transcellular pathways in tissues such as the brain have provided insight into the significance of having an alternative mechanism for breaching vascular barriers. However, the underlying regulatory process that regulates a switch between these two mechanisms remains unknown, but appears to be determined by molecules expressed by both endothelial and leucocyte populations. Furthermore, characterizing any molecular interactions that distinguish these two processes may prove to be difficult, as the molecules so far described in both processes appear not to be mutually exclusive.

The signalling pathway promoting transmigration downstream of leucocyte binding is not well characterized. It seems to implicate cytoskeleton modification, but also transcription factors such as NF-κB (nuclear factor κB) or c-Fos [72]. A better understanding of the signalling events requires further investigation.

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References


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