PECAM-1: A Multi-Functional Molecule in Inflammation and Vascular Biology

Abigail Woodfin, Mathieu-Benoit Voisin, Sussan Nourshargh

Abstract—Platelet endothelial cell adhesion molecule-1 (PECAM-1 or CD31) is a molecule expressed on all cells within the vascular compartment, being expressed to different degrees on most leukocyte sub-types, platelets, and on endothelial cells where its expression is largely concentrated at junctions between adjacent cells. As well as exhibiting adhesive properties, PECAM-1 is an efficient signaling molecule and is now known to have diverse roles in vascular biology including roles in angiogenesis, platelet function, and thrombosis, mechanosensing of endothelial cell response to fluid shear stress, and regulation of multiple stages of leukocyte migration through venular walls. This review will focus on some new developments with respect to the role of PECAM-1 in inflammation and vascular biology, highlighting the emerging complexities associated with the functions of this unique molecule. (Arterioscler Thromb Vasc Biol. 2007;27:2514-2523.)

Key Words: PECAM-1 ■ JAM-A ■ Transmigration ■ Endothelium ■ Leukocyte

Leukocyte transmigration is a hallmark of inflammatory events and is a process that is mediated and controlled at multiple levels by different adhesion pathways and activating molecules. Collectively this response involves regulated migration of leukocytes from the vascular lumen into inflamed tissues and is essential for both innate and adaptive immunity enabling tissues to respond appropriately to inflammatory stimuli such as physical injury/trauma and bacterial/viral infections. Excessive or inappropriate leukocyte accumulation in tissues can, however, contribute to a number of pathologic conditions such as atherosclerosis, vasculitis, and myocardial infarction. The process of leukocyte emigration initiates with generation of inflammatory mediators such as chemokines and cytokines and upregulation of endothelial cell adhesion molecules that can in concert support the initial attachment of leukocytes to venular endothelial cells and eventually migration through the vessel wall. This final step in the multiascade process of leukocyte emigration appears to occur largely via migration of leukocytes through junctions between adjacent endothelial cells, and numerous endothelial cell junctional molecules, such as PECAM-1, ICAM-2, JAM-A, JAM-C, ESAM, and CD99,1,6 have now been implicated in this process. The increasing number of these molecules associated with the transmigration response has raised inevitable questions regarding their multiple roles. In this context, there is now much interest and growing body of evidence indicating that distinct or multiple endothelial cell junctional molecules may mediate leukocyte transmigration.
under specific scenarios (Table 1). Within this setting, since its cloning in 1990 and the first demonstrations of its role as an adhesion molecule involved in leukocyte transendothelial cell migration in 1993, there has been tremendous progress in understanding the biology of PECAM-1 and there is now clear evidence for its involvement at multiple stages of the emigration process and the dependency of its functional role on multiple factors. The growing complexities associated with the role of PECAM-1 in regulation of leukocyte migration and inflammatory and vascular responses in certain disease models forms the basis of this brief review. It is important to note however that PECAM-1 is also implicated in numerous other biological functions including angiogenesis, apoptosis, platelet aggregation, and thrombosis, responses that are discussed more fully in recent reviews and articles.

### Brief Overview of Structure, Ligands, and Signaling

PECAM-1 is a member of the Ig gene superfamily, is composed of 6 extracellular Ig folds, has a molecular weight of 130 kDa, and is differentially glycosylated involving N-linked and O-linked glycosylation sites. Although it is generally considered that the principal ligand for PECAM-1 is PECAM-1, an interaction that can occur under both homotypic and homophilic (via domain 1) scenarios, a number of putative heterophilic PECAM-1 ligands have also been identified (Figure, A). These include a ligand expressed on transfected L cells, the integrin \( \alpha \beta \), possibly binding to PECAM-1 in cis, ADP-ribose cyclase (CD38) (domain 2), and most recently CD177 (domain 6). CD177 is expressed on a subset of neutrophils, and antibodies against this protein or domain 6 of PECAM-1 inhibit adhesion of CD177 expressing cells to immobilized PECAM-1 and inhibit transmigration across HUVEC monolayers. The in vivo relevance of such interactions on PECAM-1 functions remains unclear, and some may potentially mediate as yet uncharacterised regulatory effects on PECAM-1 expressing cells.

**Table 1. Reported Specificities Exhibited by Endothelial Cell Junctional Molecules in Mediating Leukocyte Transmigration**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Details of Specificity</th>
<th>References</th>
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<tr>
<td><strong>Leukocyte sub-type specificity</strong></td>
<td></td>
<td></td>
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<tr>
<td>PECAM-1</td>
<td>Role in transmigration of Monocytes, neutrophils and NK cells but only some sub-sets of lymphocytes</td>
<td>5, 22, 24, 33, 35, 48, 49</td>
</tr>
<tr>
<td>ESAM</td>
<td>Role in transmigration of neutrophils but not lymphocytes</td>
<td>58</td>
</tr>
<tr>
<td>CD99L2</td>
<td>Role in transmigration of neutrophils but not lymphocytes</td>
<td>57</td>
</tr>
<tr>
<td><strong>Stimulus specificity</strong></td>
<td></td>
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</tr>
<tr>
<td>PECAM-1</td>
<td>Mediates responses induced by IL-1( \beta ) but not TNF/ in the mouse cremaster muscle Mediates responses induced by IL-1( \beta ) but not HCl, adenovirus or TNF/ in mouse lungs Mediates responses induced by IL-1( \beta ), L-NAME and H2O2 but not FMLP or thrombin in rat mesentery Mediates neutrophil migration through IL-1( \beta )-stimulated ECs but not as induced by IL-8 or LTB(_4)</td>
<td>38, 44, 31, 47</td>
</tr>
<tr>
<td>JAM-A</td>
<td>Mediates responses induced by IL-1( \beta ) and I/R but not LTB(_4) or PAF or in the mouse cremaster muscle Mediates responses induced by cytokine stimulated meningitis, but not bacterial or virally stimulated meningitis in mice</td>
<td>3, 45, 46</td>
</tr>
<tr>
<td>ICAM-2</td>
<td>Mediates responses induced by IL-1( \beta ) but not TNF/ in the mouse cremaster muscle Mediates responses induced by IL-1( \beta ) but not TNF/ or thioglycollate in murine peritonitis</td>
<td>4</td>
</tr>
<tr>
<td><strong>Genetic background</strong></td>
<td></td>
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<tr>
<td>PECAM-1</td>
<td>PECAM-1 dependency noted in thioglycollate stimulated peritonitis, croton oil dermatitis and spontaneous pneumonitis in FVB/n, SJL and Swiss Webster but not C57BL/6 mice</td>
<td>48, 49</td>
</tr>
<tr>
<td><strong>Stage of migration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PECAM-1</td>
<td>Mediates leukocyte migration through ECs and the EC basement membrane and leukocyte motility</td>
<td>3, 30, 37</td>
</tr>
<tr>
<td>JAM-A</td>
<td>Can mediate leukocyte migration through ECs and mediate leukocyte motility</td>
<td>3, 6</td>
</tr>
<tr>
<td>CD99</td>
<td>Mediates migration through ECs at a stage distal to and subsequent to that mediated by PECAM-1</td>
<td>54, 55</td>
</tr>
<tr>
<td>CD99L2</td>
<td>Mediates neutrophil transmigration at the level of the EC basement membrane</td>
<td>57</td>
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EC indicates endothelial cell.
The principal structural feature of PECAM-1 involved in this context is the presence of 2 immunoreceptor tyrosine inhibitory motifs (ITIMs) in its cytoplasmic domain (for reviews see9,11). These ITIM domains can serve as docking sites for signaling molecules such as protein tyrosine phosphatases, and ligation of PECAM-1 can induce phosphorylation of tyrosine- and serine/threonine-residues of these intracellular regions. This event can lead to recruitment of molecules including the SH2-containing phosphatases (SHP-1/2, SHIP, PLC-γ1) and phospholipase C-γ1, events that can collectively lead to activation of other signaling pathways. Phosphorylation of tyrosine residues can also regulate their interaction with various cytoplasmic elements such as cytoskeletal components, eg, via recruitment or direct interaction with β and γ-catenin.9,11 Collectively such intracellular events have been associated with numerous PECAM-1–mediated responses including leukocyte transmigration, endothelial cell permeability and motility, and regulating the phenotype of cells (eg, expression/activation state of integrins).9,11 Furthermore, there is now much evidence to indicate that phosphorylation of the cytoplasmic domains of PECAM-1 can be stimulated in an out-side-in manner, eg, adherence to immobilized PECAM-1, as well as in an inside-out manner by numerous stimuli such as sheer stress, adhesion to extracellular matrix proteins (eg, fibronectin and collagen), exposure to lipopolysaccharide (LPS) and cytokines, leading to altered enhanced PECAM-1 functions.9,11,20

Regulation of Expression

PECAM-1 is expressed on the cell surface of hematopoietic and immune cells including platelets, neutrophils, monocytes, megakaryocytes, natural killer cells (NK cells), and some T cells and on endothelial cells where it is localized to the borders of adjacent cells.2,21 At present very little is known about regulation of expression of PECAM-1 on the cell surface of platelets and leukocytes, though with respect to the latter, there have been some observations of a down-regulation associated with transmigration. In vitro, although soluble inflammatory mediators were found not to alter the cell surface expression of PECAM-1 on murine neutrophils, the expression of the molecule was found to be reduced after migration through tumor necrosis factor (TNF)-α–stimulated endothelial cells.22 These findings are in line with reports on reduced expression of PECAM-1 on emigrated leukocytes in vivo into inflamed mouse corneas23 and into lymph nodes.24 Interestingly, in an in vitro model of monocyte transmigration through rat aortas, PECAM-1 expression appeared to be regulated during the transmigration process in that it was detected on monocytes above the endothelium but not below.25 Collectively these studies suggest that after transmigration, expression of PECAM-1 on emigrating leukocytes can be regulated under certain conditions, though the precise nature of this response, details of the mechanisms involved and the biological/pathological implications are at present unclear.

More information is, however, available on regulation of expression of PECAM-1 on endothelial cells. A number of studies have reported that certain cytokine combinations, ie, TNFα and IFNγ, can reduce the expression of PECAM-1 from endothelial cell junctions though the mechanism by which this occurs and its implications to leukocyte transmigration are contentious.26–28 Such a reduction in expression may occur as a result of a true downregulation of expression of PECAM-1 or redistribution of molecules away from the junctions. More recently a novel mechanism for regulation of expression of endothelial cell PECAM-1 with close associations with leukocyte transendothelial cell migration has been reported.29 Briefly, stores of PECAM-1 molecules have been shown to be concentrated in surface connected vesicular membrane invaginations at endothelial cell junctions. This
PECAM-1–rich membrane network constitutively recycles along cell borders, and during transendothelial cell migration the cycling of PECAM-1 is targeted to areas of the junction where migration occurs. Furthermore, blockade of transendothelial cell migration with anti–PECAM-1 antibodies inhibits the directed recycling of PECAM-1 but not the constitutive process. Although such studies have shed much light on regulation of expression of endothelial cell PECAM-1 under inflammatory conditions, there remain many unanswered question related to this process. Specifically, it is at present unclear under what inflammatory conditions targeted recycling of PECAM-1 occurs and the precise mechanisms that trigger this response. Furthermore, to date there exists no real-time evidence for the occurrence of such a response under flow in vitro or in vivo.

**Multiple Roles of PECAM-1 in Leukocyte Transmigration**

Although PECAM-1 was originally reported to mediate leukocyte migration through endothelial cell junctions, with no discernable role in the adhesion of leukocytes to the endothelium, it is now known to play a role in the transmigration process at multiple levels. Specifically, as well as mediating leukocyte transendothelial cell migration, there is now compelling evidence for it’s involvement in migration of leukocytes through the endothelial cell basement membrane and also in leukocyte motility.

A role for PECAM-1 in migration through cultured endothelial cells was first reported for neutrophils and monocytes and subsequently for numerous other cell types including NK cells, hematopoietic progenitor cells, and certain subsets of lymphocytes. Despite many investigations, details of the mechanisms by which PECAM-1 mediates leukocyte migration through endothelial cell junctions are unknown. It is generally considered that this response is triggered by homophilic PECAM-1 interactions at endothelial cell junctions, possibly facilitated by targeted enrichment of PECAM-1 at transmigration sites as suggested by the findings of Mamdouh et al. and mediated by PECAM-1–induced signaling events, as briefly discussed above. As well as supporting leukocyte migration through endothelial cell junctions (paracellular route), it has also been suggested that intracellular stores of PECAM-1 may contribute to the mechanisms associated with leukocyte migration through the body of the endothelium (transcellular route).

The first indications of the ability of PECAM-1 to mediate leukocyte migration through matrix proteins came from in vitro analysis of functions of different PECAM-1 domains. Briefly, using domain specific antibodies, the membrane distal region of the extracellular portion (domain 1) and the more membrane proximal region (domain 6) of the molecule were found to be involved in leukocyte migration through cultured endothelial cells and migration through the underlying collagen gel on which the endothelial cells were grown, respectively. In vivo evidence for a role for PECAM-1 in leukocyte migration through the endothelial cell basement membrane came from studies of Wakelin et al. in which an anti–PECAM-1 Ab was shown to suppress leukocyte migration through interleukin (IL)-1β–stimulated rat mesenteric venules at the level of the endothelial cell basement membrane. Further evidence for this novel function of PECAM-1 came from investigations using PECAM-1–deficient mice in which a number of models the principal defect in leukocyte emigration was noted to be migration through the endothelial cell basement membrane. Mechanism of PECAM–mediated leukocyte migration through the basement membrane is now associated with PECAM-1–mediated translocation of the integrin αβ (the principal leukocyte receptor for laminin) from intracellular stores of neutrophils to the cell surface during transendothelial cell migration.

In addition to the above there is also in vitro evidence to suggest that PECAM-1 can modulate the rate and direction of neutrophil migration, both when in contact with cytokine-activated endothelial cells and also in response to chemottractants such as FMLP. Because these observations relate to conditions involving PECAM-1/PECAM-1 interaction, the findings relate to scenarios involving interaction of leukocyte PECAM-1 with endothelial cell PECAM-1 and may well contribute to PECAM-1–mediated leukocyte transmigration. PECAM-1–deficient neutrophils have also been reported to exhibit a defect in directional motility in vitro in response to the chemokines IL-8 and KC, suggesting a role for PECAM-1 in modulating inherent neutrophil migration, an effect that maybe associated with regulation of leukocyte polarization and spreading. No such defect in chemotaxis of PECAM–deficient cells was noted in response to FMLP, and previous studies did not indicate a defect in the magnitude or rate of PECAM-1 null neutrophil migration through cultured endothelial cells in response to a gradient of IL-8, as compared with wild-type neutrophils. Hence although there exists some conflicting indications for the ability of PECAM-1 to regulate leukocyte motility in vitro, this response appears to be stimulus-specific and there exists at present no evidence for it in vivo. Findings such as the above have now led to numerous investigations into the role of PECAM-1 in leukocyte migration in vivo, studies that have largely reported on the ability of PECAM-1 blocking reagents to suppress leukocyte infiltration or leukocyte-mediated tissue damage. More recently, PECAM-1–deficient mice have also been investigated in models of inflammation but in these studies the findings have been more diverse indicating both pro- and antiinflammatory roles for PECAM-1 (Table 2). Collectively, whilst in many inflammatory models pharmacological blockade or genetic deletion of PECAM-1 results in altered leukocyte migration, often the underlying reason for the observed effects are undetermined, ie, it is unclear which of the multiple role(s) of PECAM-1 accounts for the suppressive or enhancing effects of PECAM-1 blockade/deletion.

**Role of PECAM-1 in Leukocyte Transmigration: Emerging Complexities**

There is now emerging evidence that the functional role of PECAM-1 in leukocyte transmigration is governed by multiple factors. Early studies had indicated that PECAM-1 exhibited some degree of selectivity in terms of regulating transmigration of specific leukocyte sub-types, exhibiting a significant role for monocytes and neutrophils and to a lesser
degree a role in lymphocyte and eosinophil transmigration. The ability of PECAM-1 to mediate leukocyte transmigration also appears to be stimulus-specific and can be governed by the genetic background of the experimental animal investigated (Table 1).

With respect to stimulus-specificity, data obtained by intravital microscopy studies performed in rats and mice have indicated that leukocyte emigration as elicited by local administration of IL-1β, L-NAME, and H2O2 but not TNFα, thrombin, or the chemoattractant FMLP is PECAM-1–dependent.19,31,38,43 Stimulus-specificity was also noted in a model of mouse lung injury where neutrophil infiltration into the airways as elicited by intratracheal installation of IL-1β, but not in response to TNFα, acid or adenovirus, was suppressed in PECAM-1–deficient mice.44 Using the peritonitis model, neutrophil infiltration induced by IL-1β but not thioglycollate was suppressed in PECAM-1 deficient animals.37,39 Interestingly a similar pattern of stimulus-specificity has been observed for 2 related endothelial cell junctional adhesion molecules, JAM-A and ICAM-2, both of which mediate leukocyte transmigration as induced by IL-1β but not by several leukocyte stimulating factors.34 JAM-A has also been found to have a stimulus-specific role in transmigration in models of meningitis.55–66 The underlying explanation for this stimulus specificity has yet to be determined, but one possibility is that the activation of leukocytes or the endothelium determines whether PECAM-1 (ICAM-2 or JAM-A) is recruited in mediating the transmigration response. It is potentially possible that under conditions of endothelial cell activation (eg, in response to IL-1β), leukocyte transmigration is mediated through activation/upregulation of leukocyte integrins in a PECAM-1–dependent manner. In contrast, in response to a leukocyte stimulating agent (eg, TNFα, LTB4), leukocyte integrins are directly activated and so under such conditions the need for PECAM-1–mediated integrin activation is bypassed.31,44,47

Further complexity related to the functional role of PECAM-1 was recently highlighted by the studies of Schenkel et al48,49 in which it was demonstrated that the ability of PECAM-1 to mediate leukocyte transmigration depended on the genetic background of the mice under investigation. Briefly, mice on the C57BL/6 background appeared unresponsive to PECAM-1 blockade or genetic deletion in a number of inflammatory models as compared with several other mouse strains. The reason for these findings is at present unclear but recent findings from our group indicate

### Table 2. Selected Studies Reporting Responses of PECAM-1–Deficient Mice in Models of Inflammation and Vascular Biology

<table>
<thead>
<tr>
<th>Model</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exhibit reduced inflammatory responses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thioglycollate (FVB/n strain) or IL-1β stimulated peritonitis</td>
<td>inhibition of leukocyte accumulation</td>
<td>48</td>
</tr>
<tr>
<td>IL-1β stimulated mesentery</td>
<td>delayed leukocyte transmigration, and arrest at the EC basement membrane</td>
<td>37</td>
</tr>
<tr>
<td>IL-1β stimulated cremaster muscle</td>
<td>transient delay in leukocyte migration through the EC basement membrane</td>
<td>3, 38, 39</td>
</tr>
<tr>
<td>Croton oil stimulated dermatitis (FVB/n strain)</td>
<td>inhibition of leukocyte accumulation</td>
<td>48</td>
</tr>
<tr>
<td>Foreign body inflammation</td>
<td>reduced angiogenesis and neutrophil infiltration in and around a subcutaneous polyvinyl acetyl implant</td>
<td>12</td>
</tr>
<tr>
<td>IL-1β-induced lung injury</td>
<td>inhibition of leukocyte accumulation</td>
<td>44</td>
</tr>
<tr>
<td>Pulmonary hyperoxia</td>
<td>inhibition of neutrophil accumulation</td>
<td>87</td>
</tr>
<tr>
<td>Spontaneous idiopathic pneumonitis (FVB/n strain)</td>
<td>decreased incidence of spontaneous pathology, including macrophage accumulation</td>
<td>49</td>
</tr>
<tr>
<td><strong>Atherosclerosis</strong></td>
<td>when crossed with ApoE−/− mice, exhibit reduced atherosclerotic lesion size, expression of EC adhesion molecules and leukocyte infiltration into lesions</td>
<td>personal communication with Dr K Ley.</td>
</tr>
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</table>

**Exhibit enhanced inflammatory responses**

<table>
<thead>
<tr>
<th>Model</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental auto-immune encephalopathy</td>
<td>accelerated development of clinical symptoms mononuclear cell infiltration of CNS parenchyma. Exaggerated and prolonged vascular permeability.</td>
<td>82</td>
</tr>
<tr>
<td>Septic shock</td>
<td>increased mortality, neutrophil infiltration into lungs and vascular permeability</td>
<td>84</td>
</tr>
<tr>
<td>Collagen-induced arthritis</td>
<td>enhanced incidence, accelerated on-set and severity of disease symptoms</td>
<td>89</td>
</tr>
<tr>
<td>IgE induced hypersensitivity</td>
<td>increased serum histamine and enhanced systemic and local IgE-mediated anaphylaxis</td>
<td>85</td>
</tr>
<tr>
<td>Thrombus formation</td>
<td>increased size and rate of thrombus formation</td>
<td>69</td>
</tr>
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</table>

All mice used in these studies are PECAM-1–deficient on a C57BL/6 background unless otherwise stated.
that the reported strain-specific effects may be tissue-specific. Collectively these results emphasize the need for caution in drawing generalized conclusions based on data obtained from limited experimental models. Finally it is important to note that PECAM-1 has a number of splice variants that can exhibit different adhesive properties and therefore potentially influence its ligand-binding and functional profile in leukocyte transmigration. This concept may also potentially account for variations between different mouse strains. The functional role of PECAM-1 may also be governed by differences in phenotype of endothelial cells in different vascular beds, differences in the profile of inflammatory mediators generated in different mouse strains and tissues and mechanistic differences in temporal phases of inflammatory reactions. Of relevance, it has been reported that expression levels of PECAM-1 differ in different vascular beds, being high in kidney, lung, and trachea and lower in brain, heart, and liver.

Comparative and Potential Cooperative Roles of PECAM-1 With Other Endothelial Cell Junctional Molecules

Despite the tremendous interest in dissecting the mechanisms that mediate leukocyte transmigration, very few studies have addressed the potential synergistic or cooperative interaction of different endothelial cell junctional molecules. One of the first such comparisons was between PECAM-1 and CD99. CD99 is a structurally unique 16.7-kDa type I transmembrane protein that can be heavily O-glycosylated reaching a molecular weight of 32 kDa. This molecule is expressed on the cell surface of thymocytes, T cells, neutrophils, and monocytes and has recently been shown to be expressed on endothelial cells where its expression is localized to junctions. In addition to costimulatory properties in lymphocytes and thymocytes, CD99 is now known to play a significant role in leukocyte transmigration. Specifically, the transmigration of both monocytes and neutrophils through unstimulated or cytokine-stimulated endothelial cells is significantly blocked by an anti-CD99 mAb. This effect was seen whether CD99 was blocked on leukocytes or endothelial cells, suggesting that a CD99-CD99 homophilic interaction mediates monocyte and neutrophil transmigration. CD99 blockade acted in an additive manner with PECAM-1 blockade, resulting in almost total inhibition of leukocyte transmigration. Sequential antibody blocking and confocal microscopy experiments suggested that with respect to migration through endothelial cell junctions, CD99 functions distally and subsequently to PECAM-1 though at present there is no in vivo evidence to support these in vitro findings. Recent studies have also cloned murine CD99 and found it to have 45% sequence homology with the human molecule. Furthermore through generation of anti-mouse CD99 antibodies, a role for this molecule has been found in mouse lymphocyte transendothelial cell migration in vitro and recruitment of T cells in vivo using a cutaneous delayed type hypersensitivity reaction. The functional role of a genetically and structurally related molecule, CD99 antigen-like-2 (CD99L2), also expressed on leukocytes and endothelial cells, has recently been investigated and some important differences between CD99 and CD99L2 were noted. Specifically, although both molecules mediate neutrophil transendothelial cell migration in vitro and in vivo, CD99L2, in contrast to CD99, but similar to another endothelial cell junctional molecule ESAM, does not mediate lymphocyte transmigration in a model of delayed type hypersensitivity. Furthermore, as found with PECAM-1, CD99L2 appears to block neutrophil transmigration in vivo at the level of the perivascular basement membrane, though the mechanism by which it can achieve this is at present unclear.

Studies from our group have also compared the functional role and potential additive effects of PECAM-1 with JAM-A and ICAM-2. With respect to the former, although both PECAM-1 and JAM-A were found to mediate leukocyte transmigration through mouse cremasteric venules as elicited by IL-1β, PECAM-1 mediated its effects in a homophilic manner requiring both leukocyte and endothelial cell PECAM-1 whereas JAM-A–induced transmigration only required endothelial cell JAM-A in this model. Furthermore, dual blockade or genetic deletion of PECAM-1 and JAM-A did not result in a greater level of inhibition than that seen with blockade/deletion of either molecule alone. To investigate the reason for this, the site of arrest of leukocytes in JAM-A−/− and PECAM-1−/− mice was investigated in parallel by immunofluorescence and confocal microscopy. The findings indicated that in JAM-A−/− mice, neutrophils were largely arrested at endothelial cell junctions while in PECAM-1−/− mice, in agreement with our previous findings, inhibition of neutrophil transmigration occurred at the level of the endothelial cell basement membrane. Collectively these results demonstrate that JAM-A and PECAM-1 can mediate different but sequential stages of the emigration process, namely migration through the endothelium and through the endothelial cell basement membrane, respectively. In the same model, dual blockade/genetic deletion of PECAM-1 and ICAM-2 also did not lead to a greater inhibition than that noted under conditions of blockade/genetic deletion of either molecule alone. However, this effect appeared to be tissue specific in that an anti–ICAM-2 mAb did suppress neutrophil transmigration in PECAM-1−/− mice in an IL-1β–driven peritonitis model. The reason for the observed differences in the role of ICAM-2 in PECAM-1–independent responses in different tissues is currently unclear and requires more investigations.

PECAM-1 and Vascular Disease Models

As well as investigating the role of PECAM-1 in vascular responses induced by defined inflammatory stimuli, the role of PECAM-1 has also been investigated in many inflammatory and vascular disease models some of which are discussed below.

Ischemia/Reperfusion Injury

Reperfusion of an ischemic tissue results in significant leukocyte infiltration and leukocyte-mediated tissue damage. This effect, termed ischemia/reperfusion (I/R) injury, is associated with the pathogenesis or management of numerous inflammatory conditions such as myocardial infarction and stroke, and the role of PECAM-1 in this response has been
investigated in various experimental models. These include a rat cremaster muscle model in which an anti–PECAM-1 neutralizing antibody was found to suppress I/R-induced leukocyte infiltration into tissues and stimulated microcirculatory permeability. Other models of I/R injury in which anti–PECAM-1 reagents have been shown to exert antiinflammatory effects are a rabbit hind-limb model, rat and feline models of myocardial I/R, and a rat model of intestinal I/R injury. Although in the majority of these studies anti–PECAM-1 antibodies were administered to the animals before the induction of ischemia, in the latter model of intestinal I/R injury systemic administration of an anti–PECAM-1 mAb 3 hours after initiation of the reperfusion period resulted in significant suppression of tissue neutrophil infiltration suggesting that PECAM-1 blockade may be a clinically useful mode of controlling late-stage I/R injury. A number of studies have also reported changes in cell-associated or soluble PECAM-1 after I/R injury. Collectively the current data suggest that PECAM-1 blockade can be protective in numerous models of I/R injury, and, although it is generally considered that such effects are as a result of suppressing leukocyte infiltration, PECAM-1 blockade may exert antiinflammatory effects via other modes such as effects on platelet function, though the role of PECAM-1 in this context is contentious.

Atherosclerosis

Atherosclerosis is a chronic inflammatory disease of large arteries, the pathogenesis of which involves a complex interplay between cytokines, chemokines, and adhesion molecules leading to monocyte infiltration and multiple other leukocyte responses within the arterial wall. This condition is a major cause of death in the Western world and has led to many investigations into the potential mechanisms involved. As a result of elaborate in vitro and in vivo studies it is now widely accepted that low and turbulent blood flow is a determinant of localized atherosclerotic lesions at bifurcations, branch points, and the inner curvature of large and medium-sized arteries, with high and steady laminar flow being atheroprotective. Although there have been many studies in the last few decades describing the effects of flow on endothelial cell biology, only recently have significant advancements been made with respect to the identification of molecules responsible for mechanosensing. In this context, Osawa et al reported on the possible role of PECAM-1 as a mechanoresponsive molecule. More recently, Tzima and colleagues identified PECAM-1 as a component of a mechanosensory complex comprised of PECAM-1, vascular endothelial–cadherin (VE-cadherin) and vascular endothelial growth factor receptor-2 (VEGFR2) that mediates endothelial responses to external shear stress. Within this complex, PECAM-1 was identified as the molecule that directly transmits mechanical force whereas VE-cadherin functions as an adaptor molecule and VEGFR2 activates a PI3-kinase downstream. Furthermore, although there is now clear in vivo evidence that the NF-kB signal transduction pathway in artery endothelial cells is primed for activation in regions predisposed to atherosclerotic lesion formation, branch points of aortas of PECAM-1–deficient mice showed no detectable activation of NF-kB or evidence of downstream inflammatory genes (eg, expression of intercellular adhesion molecule-1 (ICAM-1)). The role of PECAM-1 in atherosclerosis has now also been investigated in atherosclerosis prone mice, ApoE−/− animals (Personal communication with Dr K Ley 2007). The crossing of these mice with PECAM-1−/−deficient mice resulted in significantly reduced atherosclerotic lesion size, reduced expression of VCAM-1, ICAM-1, and P-selectin, and very little macrophage infiltration of atherosclerotic lesions as compared with age- and diet-matched ApoE−/− mice. Evidence was also found for reduced nuclear localization and activation of NF-kB in atheroprone regions of aortic arch in PECAM-1−/−ApoE−/− mice, as compared with ApoE−/− mice, further supporting a role for PECAM-1 in lesion development via its mechanosensory function.

In addition to the above, PECAM-1 may clearly also contribute to the pathogenesis of atherosclerosis through its ability to mediate leukocyte infiltration. Oxidized LDL has been shown to promote monocyte migration through cytokine-stimulated endothelial cells in vitro by a mechanism involving upregulation of endothelial cell PECAM-1 and downregulation of VE-cadherin. Furthermore, in the light of the fact that diabetic patients exhibit a higher incidence of atherosclerosis, it is of relevance that high glucose and insulin levels can promote increased monocyte migration through cultured endothelial cells in a PECAM-1–dependent manner, the response induced by glucose being associated with PECAM-1 phosphorylation.

Finally, there is evidence to associate certain polymorphisms in the PECAM-1 gene to elevated incidences of atherosclerosis, coronary artery disease, and myocardial infarction. The multiple functional roles and associations of PECAM-1 to atherosclerosis strongly encourage further investigations into the mechanisms that regulate the expression and function of this complex molecule in the pathogenesis of atherosclerosis and highlight the potential benefits of targeting PECAM-1 for development of novel antiatherosclerotic therapies.

Other Inflammatory and Vascular Disease Models

PECAM-1 has been implicated in the pathogenesis of numerous other inflammatory and vascular disorders including multiple sclerosis (MS), rheumatoid arthritis, sepsis, and anaphylaxis. Using models of experimental allergic encephalomyelitis (EAE), although an anti–PECAM-1 mAb was found not to effect disease on-set or the severity of clinical symptoms in rats, PECA-1–deficient mice exhibited early on-set of clinical symptoms and leukocyte infiltration during EAE. Furthermore, enhanced CNS permeability was noted in the PECAM-1–deficient mice during the development of EAE, a response that may be associated with an impairment of vascular integrity under inflammatory conditions in these animals. In humans, numerous studies have reported on enhanced levels of soluble PECAM-1 at different stages of MS and PECAM-1 expression on monocytes is elevated in patients with relapsing remitting MS as compared with control subjects. It is, however, unclear whether these observations are a cause or an effect of disease symptoms, but
it has been suggested that levels of soluble PECAM-1 may be used as a marker of disease activity. 66

PECAM-1–deficient mice also exhibited a reduced response to pulmonary hyperoxia 87 and have been investigated in models of arthritis. 83,88,89  For example, using a collagen-induced arthritis model, PECAM-1–deficient animals exhibited enhanced incidence, accelerated onset, and severity of the disease, suggesting that PECAM-1 can act as a protective molecule. 88,89  PECAM-1–deficient mice are also prone to development of other autoimmune disorders 90 and are more susceptible to vascular dysfunctions after systemic endotoxin administration. 84  Together these studies indicate that under certain inflammatory conditions, PECAM-1 has a protective role in both acute and chronic inflammatory conditions, effects that are believed to be regulated by the cytoplasmic ITIM motifs of PECAM-1, 9 though details of the mechanisms involved requires more investigation.

Concluding Remarks

In the last 15 years tremendous progress has been made in understanding the biology of PECAM-1. Specifically it is now implicated in a diverse range of responses in inflammation and vascular biology including angiogenesis, leukocyte transmigration, leukocyte motility, thrombosis, vascular permeability, and numerous immune functions. Much progress has also been made in understanding the mechanisms by which PECAM-1 mediates such diverse range of events, in particular in characterizing its expression profile, the structure of its gene, and identifying its signaling partners. Significant advancement has also been made in understanding the potential role of PECAM-1 in the pathogenesis of cardiovascular conditions such as atherosclerosis, I/R injury, and autoimmune disorders. At the same time it has become apparent that the functional role of PECAM-1 is governed by numerous factors such as the nature and tissue localization of the inflammatory response under investigation as well as genetic factors. Continued interest in understanding the functions and mechanisms of action of this complex molecule will ensure continued progress in the fields of inflammation and vascular biology as a whole.

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Disclosures

None.

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