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PECAM-1: Conflicts of Interest in Inflammation

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Abstract

Platelet endothelial cell adhesion molecule-1 (PECAM-1, CD31) is a cell adhesion and signaling receptor that is expressed on hematopoietic and endothelial cells. PECAM-1 is vital to the regulation of inflammatory responses, as it has been shown to serve a variety of pro-inflammatory and anti-inflammatory functions. Pro-inflammatory functions of PECAM-1 include the facilitation of leukocyte transendothelial migration and the transduction of mechanical signals in endothelial cells emanating from fluid shear stress. Anti-inflammatory functions include the dampening of leukocyte activation, suppression of pro-inflammatory cytokine production, and the maintenance of vascular barrier integrity. Although PECAM-1 has been well-characterized and studied, the mechanisms through which PECAM-1 regulates these seemingly opposing functions, and how they influence each other, are still not completely understood. The purpose of this review, therefore, is to provide an overview of the pro- and anti-inflammatory functions of PECAM-1 with special attention paid to mechanistic insights that have thus far been revealed in the literature in hopes of gaining a clearer picture of how these opposing functions might be integrated in a temporal and spatial manner on the whole organism level. A better understanding of how inflammatory responses are regulated should enable the development of new therapeutics that can be used in the treatment of acute and chronic inflammatory disorders.

Keywords

PECAM-1; CD31; adhesion molecules; inflammation

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Introduction

Inflammation is a multi-faceted reaction to tissue injury and/or infection. Inflammatory responses are protective; however, aberrant inflammation, whether unabated or unresolved, underlies many of the most common diseases in Western societies. Consequently, a better understanding of the biology of inflammation, and the key players involved, are vitally important to the development of treatments that prevent the undesired sequelae of inflammatory responses.

The cardinal signs of inflammation were first characterized by Celsus in the first century A.D. as *rubor* (redness), *tumor* (swelling), *calor* (heat), and *dolor* (pain) (Celsus 1935). These cardinal signs are largely the result of two main components of inflammatory responses: (1) increased vascular permeability and (2) the emigration, accumulation, and activation of leukocytes (Lawrence et al. 2002). The modulation of vascular permeability and the recruitment of leukocytes rely on cellular adhesion molecule (CAM)-mediated intercellular communication amongst adjacent endothelial cells and between endothelial cells and leukocytes. CAM-mediated interactions allow leukocytes to home to the site of inflammation, they influence the release of inflammatory mediators that activate both cell types, and they are important for the maintenance of vascular barrier function. Consequently, CAM-mediated interactions are vitally important to the initial activation, maintenance, and subsequent resolution of inflammation. PECAM-1 is one such adhesion molecule that has historically been implicated in the regulation of inflammatory responses. This review will focus on the biological properties of PECAM-1 that are pertinent to its pro- and anti-inflammatory functions.

The biology of PECAM-1

PECAM-1 is a member of the immunoglobulin (Ig)-superfamily of cell adhesion molecules. It is expressed on most cells of the hematopoietic lineage including platelets, monocytes, neutrophils, and lymphocyte subsets (Newman 1997; Newman 1999; Newman and Newman 2003). PECAM-1 is also highly expressed on endothelial cells, where it is a major constituent of the endothelial cell intercellular junction in confluent vascular beds (Muller et al. 1989; Albelda et al. 1990; Newman et al. 1990; Newman 1997).

PECAM-1 is a type I transmembrane glycoprotein that consists of an extracellular region composed of six Ig-like homology domains, a 19-residue transmembrane domain, and a 118 residue cytoplasmic tail (Newman and Newman 2003). The biological properties of PECAM-1 in cellular adhesion and signaling have been mapped to specific regions of the PECAM-1 molecule. Extracellular Ig-homology domain 1 contains residues important for mediating homophilic PECAM-1/PECAM-1 interactions (Fig. 1) (Sun et al. 1996; Newton et al. 1997). Most heterophilic binding interactions are thought to be mediated by amino acid residues located in Ig-homology domains 5 and 6 (Fig. 1). The only heterophilic binding partner of PECAM-1 that has thus far been shown to be physiologically relevant is the neutrophil-specific antigen CD177 (NB1) (Sachs et al. 2007). Other perhaps more controversial heterophilic binding partners of PECAM-1 include glycosaminoglycans (GAG) (Delisser et al. 1993; Sun et al. 1998), the integrin $\alpha_v\beta_3$ (Piali et al. 1995; Buckley et al. 1996; Sun et al. 1996), and CD38 on lymphocytes (Deaglio et al. 1998).

The cytoplasmic tail of PECAM-1 contains residues that serve as potential sites for palmitoylation, phosphorylation, and the docking of cytosolic signaling molecules (Newman and Newman 2003). The best characterized feature of the PECAM-1 cytosolic domain is two Immunoreceptor Tyrosine-based Inhibitory Motifs (ITIMs) that encompass Tyr₆₆₃ and Tyr₆₈₆ of human PECAM-1 (Fig. 1), which when phosphorylated, recruit Src homology 2 (SH2) domain-containing proteins, the best characterized of which is the SH2 domain-

containing protein tyrosine phosphatase SHP-2 (Newman and Newman 2003). Other SH2 domain-containing proteins that have been reported to associate with phosphorylated PECAM-1 ITIMs, include members of the Src family of tyrosine kinases (SFK) (Lu et al. 1997; Masuda et al. 1997; Osawa et al. 1997), SHP-1 (Hua et al. 1998; Henshall et al. 2001), SH2 domain-containing inositol 5'-phosphatase (SHIP) (Pumphrey et al. 1999), and phospholipase C γ 1 (PLC γ 1) (Pumphrey et al. 1999). Another residue in the PECAM-1 cytoplasmic domain that is subject to post-translational modification is Cys₅₉₅, which, when palmitoylated, can target PECAM-1 to membrane microdomains (Fig. 1) where it can act as a regulator of cell signaling and apoptosis (Sardjono et al. 2006).

Due to its expression on vascular and hematopoietic cells, and its signaling and adhesive capabilities, PECAM-1 is primed to serve a vital role in inflammation. Indeed, much work in recent years has implicated PECAM-1 as both a positive and negative regulator of inflammatory responses.

Pro-inflammatory roles for PECAM-1: facilitation of leukocyte transendothelial migration

After PECAM-1 was cloned and characterized in 1990 (Newman et al. 1990; Stockinger et al. 1990; Simmons et al. 1990), many of the early studies of the biological functions of PECAM-1 were focused on its pro-inflammatory role in leukocyte diapedesis. The first indications that PECAM-1 helped to promote leukocyte transendothelial migration were demonstrated in two 1993 reports showing that PECAM-1-specific antibodies blocked both leukocyte transmigration across endothelial monolayers in vitro (Muller et al. 1993) and leukocyte accumulation at sites of inflammation in vivo (Vaporciyan et al. 1993). These studies set the stage for a large body of literature further investigating the mechanism by which PECAM-1 promotes leukocyte transmigration. As such, many of the established pro-inflammatory functions of PECAM-1 (summarized in Table 1) center around its ability to support leukocyte emigration out of the vasculature and into inflammatory sites (Fig. 2).

In order to home to the site of injury or infection, leukocytes go through the well-established leukocyte adhesion cascade, which ends when they transmigrate across the endothelium in order to enter extravascular tissues in a process termed leukocyte diapedesis (Muller 2002). PECAM-1 is known to exert effects on both leukocytes and endothelial cells at both early and late stages of the leukocyte adhesion cascade (Nourshargh et al. 2006; Woodfin et al. 2007). The first part of this section will discuss how leukocyte PECAM-1 helps to promote leukocyte emigration and transmigration.

The role of leukocyte PECAM-1 in leukocyte emigration

Upstream of the leukocyte adhesion cascade, PECAM-1 on leukocytes is reported to promote chemokine-mediated directional migration of leukocytes to inflammatory sites (Wu et al. 2005). Chemokine gradients serve to direct leukocytes to their destination by activating integrins on the leukocyte surface and by promoting actin cycling and polymerization events at localized sites within the cell (Baggiolini 1998). Consequently, leukocytes that express PECAM-1 are better able to be recruited to the site of inflammation.

After leukocytes firmly adhere to the area of the endothelium to which they are recruited, they then squeeze through endothelial junctions and traverse the perivascular basement membrane to enter the inflamed tissue, both of which are processes that PECAM-1 is known to promote. One of the well-established mechanisms by which PECAM-1 promotes these processes is through homophilic PECAM-1/PECAM-1 adhesive interactions between leukocytes and endothelial cells as they traverse the endothelial cell-cell junction (Muller et al. 1993; Liao et al. 1995; Liao et al. 1997; Nakada et al. 2000). Though GAGs and the

integrin $\alpha_v\beta_3$ expressed on endothelial cells have been reported as heterotypic ligands for *leukocyte* PECAM-1 (Delisser et al. 1993; Piali et al. 1995; Buckley et al. 1996), their physiological relevance remains in question (Sun et al. 1996; Sun et al. 1998). Physiologically relevant heterotypic ligands for *endothelial* PECAM-1 have, however, been shown to be expressed on leukocytes (Sachs et al. 2007), and as such, they will be discussed in the next section.

Homophilic PECAM-1/PECAM-1 interactions are not only thought to play an important adhesive role, but they are also thought to trigger signaling events that lead to the activation of integrins on leukocytes. Leukocyte integrins reported to be activated downstream of PECAM-1 ligation include the β_1 - and β_2 -integrins in T cell subsets (Tanaka et al. 1992), β_1 -integrins in macrophages (Vernon-Wilson et al. 2006; Vernon-Wilson et al. 2007), β_2 -integrins in natural killer (NK) cells (Berman et al. 1996), and the $\alpha_M\beta_2$ (Mac-1, CD11b/CD18) integrin in monocytes and neutrophils (Berman and Muller 1995). Additionally, homophilic PECAM-1 interactions trigger the upregulation of the integrin $\alpha_6\beta_1$ – a receptor for laminin, a major component of the basal lamina (Dangerfield et al. 2002) – on neutrophils, which enables them to traverse the perivascular basement membrane (Dangerfield et al. 2002; Wang et al. 2005). In fact, leukocytes that lack PECAM-1 are not able to efficiently transmigrate and are restrained between the endothelium and perivascular basement membrane (Liao et al. 1995; Duncan et al. 1999; Thompson et al. 2001; Woodfin et al. 2009). Thus, one of the main functions of PECAM-1 in leukocytes is to activate integrins downstream of homophilic ligation, which enables the leukocyte to completely transmigrate through the endothelium and subendothelial matrix.

Though the process by which PECAM-1 promotes integrin activation in leukocytes is still poorly understood, there have been hints at potential mechanisms. Antibody-mediated ligation of PECAM-1 in Jurkat T cells was reported to activate Rap1, a Ras family GTPase, and this activation required the presence of functional ITIMs in the cytoplasmic tail of PECAM-1 (Reedquist et al. 2000). Following activation, Rap1 is known to be vital to the control of cell-adhesive functions as it promotes phagocytosis, cell migration and spreading, and inside-out integrin activation (Caron 2003). In a second study, a functional association between PECAM-1 and phosphoinositide-3 kinase (PI3K) was reported in neutrophils, and blocking the PI3K pathway with pharmacologic inhibitors downstream of PECAM-1 ligation prevented neutrophil adhesion to fibronectin or to fibroblasts transfected with ICAM-1 (Pellegatta et al. 1998). Additionally, it was demonstrated that PECAM-1 monomers, dimers, and oligomers exist on the cell surface in a dynamic equilibrium, and that PECAM-1 best serves as a positive activator of integrins when oligomerized (Zhao and Newman 2001). Finally, PECAM-1 was shown to inhibit membrane repolarization mediated by the ether-a-go-go related gene (ERG) voltage gated potassium channel, which was correlated with enhanced β_1 -integrin-mediated firm adhesion of phagocytes to apoptotic cells (Vernon-Wilson et al. 2007). Thus, it seems that PECAM-1 promotes integrin activation through: (1) the regulation of other integrin modulators, (2) its association with other PECAM-1 molecules within the plane of the membrane, and/or (3) the modulation of cell membrane potential.

Taken together, these studies support an important pro-inflammatory role for leukocyte PECAM-1 in leukocyte extravasation to sites of injury and inflammation, as it helps to promote integrin activation in leukocytes downstream of homophilic adhesive interactions that involve endothelial PECAM-1. PECAM-1-dependent integrin activation then enables leukocytes to migrate through both the endothelial junction and subendothelial basement membrane to enter extravascular sites.

The role of endothelial PECAM-1 in leukocyte emigration

Much like in leukocytes, adhesive interactions are a main mechanism through which endothelial PECAM-1 helps to facilitate leukocyte transmigration. Homophilic PECAM-1/PECAM-1 interactions between endothelial cells and leukocytes were thought to be the only physiologically relevant binding interaction in which endothelial PECAM-1 participated until the recent discovery of CD177 as a bona fide neutrophil heterophilic binding partner of endothelial PECAM-1 (Sachs et al. 2007). The heterophilic interaction between neutrophil CD177 and endothelial PECAM-1 is functionally relevant as blocking antibodies directed against either CD177 or Ig-domain 6 of endothelial PECAM-1 were able to significantly inhibit neutrophil transmigration toward chemotactic gradients, and CD177-positive neutrophils migrated more rapidly than CD177-negative neutrophils (Sachs et al. 2007). It should be noted, however, that CD177 is only expressed on ~45-65% of any individual's neutrophils, and that 3-5% of people do not express CD177 at all, with no readily apparent inflammatory defect (Stroncek et al. 1996; Matsuo et al. 2000). Since CD177 has been shown to be upregulated in severe bacterial infections (Gohring et al. 2004), after granulocyte-colony stimulating factor (G-CSF) stimulation (Gohring et al. 2004), and in pathological conditions in newborns (Wolff et al. 2006), it remains to be determined whether the CD177/PECAM-1 transmigration pathway is utilized preferentially in certain inflammatory states by neutrophils that express CD177. At any rate, endothelial PECAM-1 facilitates leukocyte transmigration through two different adhesive mechanisms: (1) homophilic interactions with leukocyte PECAM-1 on neutrophils, monocytes, and lymphocytes, and (2) heterophilic interactions with neutrophil CD177. The identification of CD177 on neutrophils as a heterotypic binding partner of endothelial PECAM-1 raises the intriguing possibility that distinct heterotypic binding partners on other leukocyte subsets act to support transendothelial migration. Additionally, it remains to be determined how ligation of endothelial PECAM-1 by neutrophil CD177 modulates signaling within endothelial cells to promote leukocyte transmigration, and also if heterotypic CD177/PECAM-1 interactions promote integrin activation in leukocytes as do homotypic interactions (Tanaka et al. 1992; Berman and Muller 1995; Berman et al. 1996; Dangerfield et al. 2002; Wang et al. 2005). These remain active areas of investigation.

A prominent mechanism by which endothelial PECAM-1 helps to support leukocyte transmigration was revealed in a series of elegant studies that identified PECAM-1 as a main constituent of a recycling compartment on endothelial cells, termed the lateral border recycling compartment (LBRC). The LBRC is a surface-connected membrane network, distinct from caveolae or vesiculo-vacuolar organelles (VVO), that is located at the borders between adjacent endothelial cells (Mamdouh et al. 2003). This membrane network contains PECAM-1, CD99, and junctional adhesion molecule (JAM)-A, and is recycled and targeted to the region of the cell where paracellular or transcellular migration is occurring (Mamdouh et al. 2003; Mamdouh et al. 2009). Interestingly, when monocyte PECAM-1 was blocked with an anti-PECAM-1 blocking antibody, monocytes adhered to endothelial cells and moved to endothelial junctions normally but were unable to transmigrate, and endothelial PECAM-1 was not targeted to the zone around the monocyte (Mamdouh et al. 2003). Consequently, homophilic PECAM-1/PECAM-1 interactions between leukocytes and endothelial cells are important for triggering targeted PECAM-1- and LBRC-recycling, which is required for leukocyte transmigration (Mamdouh et al. 2003). Later work by this same group demonstrated that both PECAM-1-dependent and PECAM-1-independent leukocyte transmigration is dependent on LBRC recycling mediated by endothelial microtubules and kinesin family molecular motors (Mamdouh et al. 2008). Interestingly, only Y₆₆₃ of the PECAM-1 cytoplasmic ITIM, but not Y₆₈₆ nor ITIM-mediated recruitment of SHP-2, is essential for PECAM-1 to efficiently enter and exit the LBRC and to support targeted recycling of the LBRC (Dasgupta et al. 2009).

Although endothelial cell expression of PECAM-1 is clearly important for leukocyte transendothelial migration, it is not clear whether signal transduction events mediated by the cytoplasmic domain of endothelial PECAM-1 are required for these processes. Endothelial PECAM-1 engagement may trigger signaling pathways that lead to a rise in intracellular calcium ion concentration (Gurubhagavatula et al. 1998; O'Brien et al. 2001), which helps initiate signaling pathways that open up intercellular junctions and allow leukocytes to transit across the endothelial monolayer (Huang et al. 1993). On the other hand, O'Brien, et al. reported that leukocyte transmigration might not be dependent on signaling by endothelial PECAM-1 as endothelial-like REN cells expressing mutant forms of PECAM-1 lacking the entire cytoplasmic domain or portions of the cytoplasmic domain known to be important for PECAM-1-mediated signaling in other cells were still able to support leukocyte transmigration (O'Brien et al. 2003). Taken together, this study, along with the study by Dasgupta, et al. described in the preceding paragraph, indicate that ITIM-mediated recruitment of SHP-2 in endothelial cells does *not* appear to be required for leukocyte transmigration (O'Brien et al. 2003; Dasgupta et al. 2009). Since PECAM-1-dependent leukocyte transmigration requires Y₆₆₃-mediated targeted recycling of PECAM-1 and the LBRC (Mamdouh et al. 2003; Mamdouh et al. 2008; Mamdouh et al. 2009), however, it is likely that this residue or other residues within the cytoplasmic tail of PECAM-1 serve as docking sites for currently uncharacterized signaling partners and/or as targeting motifs that result in the trafficking of PECAM-1 to the LBRC. These remain active areas of investigation.

Whereas the studies described above demonstrate an important role for PECAM-1 in leukocyte diapedesis, it is important to point out that the requirement for PECAM-1 in this process is stimulus dependent. In vivo, PECAM-1 has been shown to be required for leukocyte transmigration in response to certain stimuli, such as IL-1 β , but not TNF α nor certain chemokines (Thompson et al. 2001; Woodfin et al. 2009). It has been suggested that stimuli such as IL-1 β , which mainly activate endothelial cells as opposed to leukocytes, render leukocytes dependent on transmigration mediated by ICAM-1, JAM-A, and PECAM-1 in a sequential manner (Nourshargh et al. 2006; Woodfin et al. 2007; Woodfin et al. 2009). Other stimuli, such as TNF α and chemokines, may bypass the need for these adhesive interactions by directly activating the leukocyte and allowing it to transmigrate through junctions (Woodfin et al. 2009). The stimulus dependence of the requirement for PECAM-1 in leukocyte transendothelial migration lends further support to the importance of PECAM-1-dependent adhesive interactions for integrin activation in leukocytes, as it appears that certain stimuli cannot fully activate leukocyte integrins and thus rely on PECAM-1-mediated integrin activation to support complete leukocyte transendothelial migration.

The evidence for PECAM-1's role in leukocyte transmigration set the precedent for later studies demonstrating that PECAM-1 blocking reagents might be clinically beneficial for blockade of leukocyte emigration in inflammatory diseases (Table 1). Antibodies directed against PECAM-1 blocked accumulation of neutrophils in (1) the peritoneum following glycogen-induced peritonitis in mice and rats, (2) the lung following IgG immune complex deposition in rats, and (3) human skin grafts transplanted onto immunodeficient mice (Vaporciyan et al. 1993; Bogen et al. 1994). Additionally, PECAM-1 blocking reagents attenuated disease progression in a model of endotoxin-induced keratitis (Khatri et al. 2002); decreased disease burden in dextran sulfate sodium (DSS)-induced colitis (Rijcken et al. 2007), which is a murine model of inflammatory bowel disease; significantly reduced ischemia-reperfusion injury in rats by preventing the accumulation of neutrophils in the myocardium following ischemic injury (Gumina et al. 1996); attenuated the severity of experimental autoimmune encephalitis (EAE) following short-term administration (Reinke et al. 2007); and significantly eliminated cartilage and bone destruction in collagen

antibody-induced arthritis (Dasgupta et al. 2010). Taken together, these studies indicate the important pro-inflammatory role of PECAM-1 in leukocyte emigration to sites of injury and inflammation, while potentially demonstrating the efficacy of PECAM-1 blocking reagents in inflammatory disorders.

Anti-inflammatory roles for PECAM-1

On the basis of its role in leukocyte transmigration alone, it would seem that PECAM-1 mainly functions to support the inflammatory process; however, it is becoming apparent that perhaps a more dominant function of PECAM-1 is to suppress inflammatory responses. More specifically, PECAM-1 has been found to dampen inflammation in a variety of clinically-relevant acute and chronic inflammatory conditions in C57BL/6 mice (summarized in Table 2), including collagen-induced arthritis (Tada et al. 2003; Wong et al. 2005), late-stage autoimmunity (Wilkinson et al. 2002), autoimmune encephalitis (Graesser et al. 2002), lipopolysaccharide (LPS)-induced endotoxic shock (Maas et al. 2005; Carrithers et al. 2005), atherogenic diet-induced steatohepatitis (Goel et al. 2007), and atherosclerosis (Goel et al. 2008). PECAM-1 is thought to exert its anti-inflammatory effects through three main mechanisms, including: (1) raising the threshold for leukocyte activation as a consequence of its function as an inhibitory receptor (Newton-Nash and Newman 1999; Newman et al. 2001; Wilkinson et al. 2002; Wong et al. 2002; Rui et al. 2007), (2) helping to maintain and restore the vascular barrier (Graesser et al. 2002; Carrithers et al. 2005; Maas et al. 2005), and (3) dampening production of pro-inflammatory cytokines (Tada et al. 2003; Carrithers et al. 2005; Maas et al. 2005; Goel et al. 2007). These three mechanisms will be discussed below.

The inhibitory role of PECAM-1 in leukocyte activation

Some of the earliest studies that implicated PECAM-1 as a possible inflammation-dampening receptor were centered around the ability of PECAM-1 to raise the threshold for leukocyte activation through its cytoplasmic ITIMs. The first of these studies demonstrated that PECAM-1 is able to dampen T cell activation through attenuation of calcium mobilization from intracellular stores (Newton-Nash and Newman 1999). It was later revealed that reduction of calcium mobilization in B cells by PECAM-1 cross-linking required the PECAM-1 ITIMs and the presence of SHP-2 (Newman et al. 2001). These studies helped to demonstrate that PECAM-1 could indeed function as an inhibitory receptor and were the first to provide evidence for inclusion of PECAM-1 in the ITIM family. In support of PECAM-1 as an inhibitory receptor in lymphocytes, PECAM-1^{-/-} mice exhibit aberrant proliferation and activation of B cells, which correlates with development of autoimmune disease in older mice (Wilkinson et al. 2002).

PECAM-1 inhibitory receptor functions are not restricted to lymphocytes, but appear to apply to mast cells and macrophages as well. PECAM-1 suppresses mast cell activation, which prevents systemic and local IgE-dependent anaphylactic reactions when animals are challenged with allergic stimuli (Wong et al. 2002). In macrophages, ligation of PECAM-1 with a CD38-Fc fusion protein (a reported heterotypic ligand for PECAM-1 on lymphocytes) was reported to negatively regulate Toll-like receptor (TLR) 4 signaling, likely through ITIM/SHP-2 interactions (Rui et al. 2007). Taken together, these studies provide compelling evidence that PECAM-1 is able to negatively regulate pro-inflammatory activation in lymphocytes, mast cells, and macrophages, likely through ITIM-mediated inhibitory signaling (Fig. 3).

PECAM-1 helps to maintain vascular barrier function

The first indication that PECAM-1 was important for maintenance of the vascular barrier was demonstrated by Ferrero, et al., who showed that a blocking antibody directed against PECAM-1 increased the permeability of endothelial monolayers in vitro and of multiple vascular beds in vivo (Ferrero et al. 1995). It was further demonstrated that PECAM-1 helps to promote vascular barrier function in response to a wide range of inflammatory stimuli. In EAE, which is an established rodent model of the human disease multiple sclerosis, mice expressing PECAM-1 had less inflammatory cell infiltration into the brain parenchyma, and this phenotype correlated with enhanced barrier function of PECAM-1-expressing endothelial monolayers (Graesser et al. 2002). Expression of PECAM-1 also hastened restoration of the vascular barrier in LPS-induced endotoxemia, a well-established mouse model of sepsis (Carrithers et al. 2005; Maas et al. 2005). Additionally, PECAM-1-deficient, relative to PECAM-1-expressing, endothelial monolayers are hyperpermeable to histamine both in vivo (Graesser et al. 2002) and in vitro (Graesser et al. 2002; Biswas et al. 2006).

The precise mechanism(s) by which PECAM-1 helps to preserve and restore vascular integrity are still poorly understood. It is possible that PECAM-1 helps to maintain vascular barrier function through modulation of other primary regulators of permeability. One mechanism might be through interactions with catenins, which are proteins known to enhance barrier function (Komarova et al. 2007). PECAM-1 has been reported to bind, maintain the de-phosphorylated (barrier protective) state, and enhance the stability of catenins, which has been correlated with PECAM-1-mediated vascular barrier enhancement (Matsumura et al. 1997; Biswas et al. 2003; Biswas et al. 2005; Biswas et al. 2006).

An alternative, and potentially attractive mechanism for PECAM-1-mediated barrier maintenance is via modulation of signaling by sphingosine-1-phosphate (S1P), which is known to promote vascular barrier function through adherens junction assembly (Komarova et al. 2007). Sphingosine kinase (Sphk) is the upstream kinase that produces active S1P (Rosen and Goetzl 2005). PECAM-1 has been shown to interact with Sphk in transfected cells and modulate its function (Fukuda et al. 2004; Limaye et al. 2005), suggesting that PECAM-1 might regulate S1P signaling upstream of S1P binding to its cell surface receptors. Localization of S1P receptors to membrane microdomains has been found to be important for their signaling functions (Igarashi and Michel 2000). PECAM-1 might regulate S1P function at the receptor level as it has been shown to modulate signaling from receptors localized to membrane microdomains (Sardjono et al. 2006; Lee et al. 2006). Downstream of its cell surface receptor, S1P was reported to induce the phosphorylation of PECAM-1 in a $G\alpha_i$ - and SFK-dependent manner, though S1P-mediated phosphorylation of PECAM-1 was not correlated with enhanced vascular barrier function (Huang et al. 2008). Due to the importance of S1P in vascular barrier function and the ability of S1P and PECAM-1 to regulate each other, further clarity is needed to determine whether PECAM-1 can indeed promote barrier maintenance through S1P signaling.

One other interesting mechanism by which PECAM-1 could promote barrier function is through Rap1. Rap1 is important for restoring barrier function in endothelial cells through accelerated assembly of endothelial cell-cell junctions (Wittchen et al. 2005). If PECAM-1 promotes activation of Rap1 in endothelial cells, as it does in T cells (Reedquist et al. 2000), junctional assembly would be accelerated resulting in enhanced barrier function. Further work needs to be done to determine whether any or all of these mechanisms are important for PECAM-1-mediated vascular barrier maintenance. Identifying the pertinent biological properties of PECAM-1 (i.e. homophilic adhesion, localization to lipid rafts, ITIM-mediated signaling) that help to promote vascular barrier maintenance will go a long way toward answering these questions.

PECAM-1 dampens cytokine production

Studies conducted in our laboratory and by Carrithers, et al. established that PECAM-1 helps to suppress the production of pro-inflammatory cytokines following endotoxin exposure (Maas et al. 2005; Carrithers et al. 2005). It was subsequently demonstrated that PECAM-1 also suppresses cytokine production in two other mouse models of inflammation, namely nonalcoholic steatohepatitis (Goel et al. 2007) and collagen-induced arthritis (Tada et al. 2003). In the latter study, lymphocytes expressing PECAM-1 produced lower levels of the pro-inflammatory cytokine, IFN γ , following stimulation with collagen than did PECAM-1^{-/-} lymphocytes (Tada et al. 2003). Taken together, these studies firmly establish that expression of PECAM-1 is important for dampening levels of multiple pro-inflammatory cytokines on the cellular and whole animal level.

The mechanism by which PECAM-1 regulates cytokine production is still poorly understood. Rui, et al. have been the only group to reveal a potential mechanism through which PECAM-1 dampens cytokine production. They reported that PECAM-1 ligation in T cells with a CD38 fusion protein was able to inhibit activation of the JNK, NF- κ B, and IRF-3 pathways, which was correlated with dampened cytokine production (Rui et al. 2007). Since these signaling pathways regulate a variety of signaling pathways within cells, greater clarity is needed as to the mechanism by which PECAM-1 might inhibit these pathways. It will also be important in future studies to determine the main cellular source of pro-inflammatory cytokines that PECAM-1 inhibits to exert its anti-inflammatory effects. Studies using leukocytes and endothelial cells isolated from PECAM-1^{+/+} and PECAM-1^{-/-} mice will go a long way toward clarifying PECAM-1's role in regulating cytokine production.

Endothelial PECAM-1 exerts the predominant protective effects in inflammation

One additional insight into the protective effects of PECAM-1 in inflammation has been gleaned from studies of bone marrow chimeric mice that express PECAM-1 selectively on either endothelial cells or leukocytes. These studies have revealed that endothelial, but not leukocyte, PECAM-1, is largely sufficient for protection against excessive inflammation in the inflammatory disease models EAE (Graesser et al. 2002) and LPS-induced endotoxemia (Maas et al. 2005). The specific mechanism(s) by which endothelial PECAM-1 is protective in these disease models, however, is not completely understood. PECAM-1 is likely to be protective in endothelial cells during inflammation due to its ability to (1) inhibit cytokine production, (2) maintain vascular integrity, and/or (3) inhibit pro-inflammatory signaling. Since the first two mechanisms have been previously discussed, we will now focus our attention on the third.

One mechanism that helps to explain some of the anti-inflammatory effects of endothelial PECAM-1 is PECAM-1-mediated promotion of signal transducer and activator of transcription 3 (STAT3) signaling in endothelial cells (Carrithers et al. 2005). STAT3 is known to be important in the regulation of the acute phase response during inflammation (Alonzi et al. 2001), and although it can mediate transcription of both pro- and anti-inflammatory genes, studies in cell-type specific knockout mice suggest that its anti-inflammatory effects might predominate in models of inflammation (Takeda et al. 1999; Kano et al. 2003). Expression of PECAM-1 was correlated with enhanced phosphorylation of STAT3 in both endothelial cells and splenocytes from mice (Carrithers et al. 2005). On this basis, it has been proposed that binding of SHP-2 to the PECAM-1 ITIMs sequesters SHP-2 away from STAT3, which prevents SHP-2-mediated STAT3 dephosphorylation and prolongs activation of STAT3 (Carrithers et al. 2005). Consequently, endothelial cells expressing PECAM-1 are postulated to have more STAT3 mediated anti-inflammatory signaling. This mechanism bears further examination in mice, however, since the

predominant isoforms of PECAM-1 that are expressed in murine tissues, including endothelial cells, lack exon 14 (contains the second cytoplasmic ITIM) (Sheibani et al. 1997; Sheibani et al. 1999; Wang and Sheibani 2002), and thus are not likely to be able to efficiently recruit SHP-2 (Wang and Sheibani 2006; Dimaio and Sheibani 2008).

Alternatively, Cepinskas, et al. reported that PECAM-1 engagement, induced by either antibody mediated cross-linking or leukocyte transmigration, resulted in decreased levels of NF- κ B in the nuclei of endothelial cells, which led the authors to propose that inhibition of NF- κ B translocation to the nucleus by PECAM-1 initiates a negative feedback loop that prevents excessive leukocyte recruitment to sites of inflammation by dampening the NF- κ B-dependent expression of pro-inflammatory adhesion molecules on the endothelial cell surface (Cepinskas et al. 2003). In a series of extensive investigations, however, we have been unable to confirm this mechanism of PECAM-1-mediated anti-inflammatory signaling (Privratsky et al. 2010). Using a variety of antibody reagents to cross-link PECAM-1 in primary endothelial cells, we found that neither engagement nor cross-linking of PECAM-1 has an inhibitory effect on NF- κ B activity, as determined by Western blot analysis for phosphorylated and total I κ B α , immunofluorescence for detection of nuclear NF- κ B, or electrophoretic mobility shift assays to detect binding of NF- κ B to target oligonucleotides (Privratsky et al. 2010). We also found that higher levels of PECAM-1 expression do not correlate with lower levels of NF- κ B transcriptional activity in cytokine-stimulated HEK293 cells containing an NF- κ B luciferase reporter plasmid, nor do they prevent the upregulation of ICAM-1, an NF- κ B target gene, in cytokine-stimulated endothelial cells (Privratsky et al. 2010). Taken together, these results suggest that the anti-inflammatory effects of PECAM-1 in endothelial cells, at least in the case of inflammatory mediators such as LPS and cytokines, are likely not due to inhibition of NF- κ B transcriptional activity.

Even though engagement of endothelial PECAM-1 during leukocyte transmigration does not appear to inhibit NF- κ B activity, it likely does send inhibitory signals to prevent excessive endothelial activation. Couty, et al. demonstrated that PECAM-1 ligation with monoclonal antibodies counteracted ICAM-1 ligation-induced endothelial activation and cytoskeletal rearrangement, which is thought to promote junctional opening and leukocyte transit (Couty et al. 2007). These results would suggest that as leukocytes transmigrate through the junction, PECAM-1 becomes engaged, which then signals for the endothelial cell to close the junction and return to the basal state (Couty et al. 2007). This attractive hypothesis also has implications for PECAM-1-mediated vascular barrier protection as cytoskeletal rearrangements are prominent during barrier disruption.

The interplay of the pro- and anti-inflammatory functions of PECAM-1

Though seemingly opposing pro- and anti-inflammatory roles for PECAM-1 have been established, which of these roles dominates is likely to depend on the context of the cells, organs, inflammatory stimulus, and animal model used. Studies examining PECAM-1's role in atherosclerotic development, how strain-specific differences confer PECAM-1-independent leukocyte transmigration, and the biological outcomes resulting from the differential expression of alternatively-spliced PECAM-1 isoforms in mouse and human tissues have provided some insights into how the pro- and anti-inflammatory properties influence each other spatially and on the whole animal level.

PECAM-1 as site-specific regulator of atherosclerotic lesion development

Though most studies demonstrating a pro-inflammatory role for PECAM-1 have centered around the process of leukocyte emigration, there is a growing body of literature in the field of atherosclerosis research revealing that PECAM-1 promotes development of inflammatory responses through another mechanism, that being as a mechanosensor that helps to activate

endothelial cells in response to mechanical stimulation (Fig. 2). One of the main components of atherosclerotic lesion development is the response of the vessel to the flowing blood within it. The endothelium is constantly subjected to mechanical forces such as stretch, cyclic mechanical strain, and fluid shear stress (Lehoux et al. 2006). Signals emanating from these forces are thought to be transmitted by the cytoskeleton from the apical surface of the endothelial cell to points of attachment at cell-cell and cell-matrix junctions (Tzima et al. 2005). Such signals can be pro-inflammatory by inducing expression of adhesion molecules that support leukocyte adhesion and transmigration, and the nature of the mechanical forces to which endothelial cells are exposed determine whether signal transduction is initiated or not. Endothelial cells adapt to unidirectional, or laminar, shear stresses and therefore fail to activate pro-inflammatory signaling pathways (Davies 1997; Tzima et al. 2005). As such, unidirectional or laminar shear stress is thought to be atheroprotective (Davies 1997). In contrast, in regions of oscillatory or disturbed flow, which occur at vessel bifurcations and in regions of high curvature, endothelial cells are unable to adapt to shear stresses and therefore activate pro-inflammatory signaling pathways without compensatory downregulation (Mohan et al. 1997). As a result, atherosclerotic lesions tend to develop preferentially in these regions of low or disturbed shear stress (Davies 1997).

Much work has gone into identifying the cell surface receptors that transmit mechanical signals. Few “mechano-responsive” receptors have been identified, however, and the mechanisms by which these receptors transmit signals are largely unknown. It is thought that components of the cell-cell and cell-matrix junctions are candidates to transduce these signals, and due to PECAM-1’s localization to cell-cell junctions in endothelial cells, it emerged as a prime candidate. The first studies examining PECAM-1 as a mechanosensor reported that PECAM-1 becomes tyrosine phosphorylated by SFK in response to mechanical force (Osawa et al. 1997), which allows it to recruit SHP-2 and subsequently activate extracellular signal-regulated kinase (ERK) (Osawa et al. 2002). Further proof that PECAM-1 is part of a mechanosensory complex that responds to shear stress on endothelial cells was demonstrated by Tzima, et al (Tzima et al. 2005). The authors of this study found that VE-cadherin and PECAM-1 cooperate in endothelial cells to induce PI3K/Akt-mediated integrin activation, align actin filaments, and activate NF- κ B following shear stress (Tzima et al. 2005). Responsiveness to flow in endothelial cells was further shown to be dependent on (1) PECAM-1, which transmits mechanical force, (2) VE-cadherin, which functions as an adaptor, and (3) VEGFR2, which activates PI3K (Tzima et al. 2005). PI3K subsequently activates NF- κ B, causing pro-inflammatory gene induction. Accordingly, mice that express PECAM-1 are able to transduce signals in response to mechanical force and activate NF- κ B, which results in the transcription of pro-inflammatory genes at regions of disturbed flow (Tzima et al. 2005) and subsequently induces vascular remodeling (Chen and Tzima 2009). PECAM-1-mediated promotion of atherosclerotic lesion development was further confirmed in two other independent studies (Harry et al. 2008; Stevens et al. 2008).

In contrast, another study has demonstrated that PECAM-1 can have site-specific *atheroprotective* effects during the development of atherosclerosis. LDL receptor knockout (LDL^{-/-})/PECAM-1^{+/+} mice that are fed a high fat diet were shown to have significantly decreased atherosclerotic lesion area in the total aorta with preferential protection in the aortic sinus, descending aorta, and the branching arteries of the aortic arch compared to LDL^{-/-}/PECAM-1^{-/-} mice (Goel et al. 2008). Interestingly, in support of the studies described above (Tzima et al. 2005; Harry et al. 2008; Stevens et al. 2008), expression of PECAM-1 did, however, promote atherosclerotic development in the inner curvature of the aortic arch, an area associated with disturbed flow (Goel et al. 2008).

Taken together, the results of these studies indicate that PECAM-1 can have *atheroprotective* effects in certain areas of the vasculature (Goel et al. 2008), but *pro-*

atherosclerotic effects in other areas of the vasculature (Tzima et al. 2005; Harry et al. 2008; Stevens et al. 2008; Goel et al. 2008). These seemingly contradictory results can likely be explained by the different biological functions of PECAM-1. Pro-atherosclerotic effects of PECAM-1 in the inner curvature of the aortic arch support the concept that PECAM-1 acts as a mechanotransducer that contributes to the development of atherosclerosis in regions of disturbed shear stress (Tzima et al. 2005; Harry et al. 2008; Stevens et al. 2008; Goel et al. 2008). In these areas of disturbed flow and shear, the function of PECAM-1 as a mechanotransducer appears to be required, perhaps to both induce NF- κ B-dependent expression of pro-inflammatory adhesion molecules and enable leukocyte transmigration into the affected region, both of which would be pro-inflammatory and pro-atherosclerotic. In contrast, in other regions of the vasculature (descending aorta, aortic sinus, branching vessels), where PECAM-1 was demonstrated to have atheroprotective effects (Goel et al. 2008), the role of PECAM-1 in mechanotransduction and leukocyte transmigration do not appear to be as important and its anti-inflammatory properties (i.e. the maintenance of vascular barrier function and dampening of pro-inflammatory cytokine production) predominate, which slows the development of atherosclerotic lesions. Future work will need to be aimed at reconciling these differences, and definitively determining which biological function of PECAM-1 predominates at what time and in which area. It is also interesting to note that certain single nucleotide polymorphisms (SNP) within PECAM-1 have been associated with a higher risk of coronary artery disease, which is a downstream consequence of atherosclerosis (Wei et al. 2004). It will be interesting to determine whether these SNPs change the biological properties of PECAM-1 such that it is better able to transduce mechanical signals or less well able to exert anti-inflammatory effects. It is also currently not known how PECAM-1 converts mechanical signals into cellular signals, or how it couples to VE-cadherin and VEGFR2 signaling. One potential mechanism could be through association with G-protein coupled receptors (GPCR), such as the bradykinin receptor B2 (Yeh et al. 2008; Otte et al. 2009), through which PECAM-1 could modulate VE-cadherin/VEGFR2 signaling.

Strain-specific differences confer PECAM-1-independent leukocyte transendothelial migration

The important roles that PECAM-1 plays in recruiting leukocytes to sites of inflammation and supporting their extravasation support the hypothesis that a PECAM-1-deficient mouse might exhibit severe defects in leukocyte trafficking. Interestingly, when Duncan, et al. generated the first PECAM-1 knockout mice on the C57BL/6 genetic background, they unexpectedly found that normal numbers of leukocytes were recovered from sites of inflammation in these mice, with the only phenotype being trapping of leukocytes at the perivascular basement membrane (Duncan et al. 1999). To further investigate these unexpected findings, Schenkel, et al. backcrossed C57BL/6 PECAM-1 knockout mice onto the FVB/n strain, and they showed that these mice *did* display defects in leukocyte emigration following thioglycollate-induced peritonitis and croton oil-induced topical dermatitis (Schenkel et al. 2004). They further demonstrated that leukocyte emigration could be blocked by anti-PECAM-1 reagents in not only FVB/n, but also SJL and Swiss Webster mice, whereas emigration could not be blocked by these same reagents in C57BL/6 PECAM-1^{-/-} mice (Schenkel et al. 2004). As such, C57BL/6 PECAM-1^{-/-} mice are unique among mouse strains in their ability to compensate for loss of PECAM-1 function in leukocyte transmigration (Schenkel et al. 2004). QTL mapping between PECAM-1^{-/-} FVB and C57BL/6 mice has subsequently identified a single locus on chromosome 2 that confers PECAM-1-independent leukocyte transmigration in C57BL/6 mice, though the specific gene(s) remain to be determined (Seidman et al. 2009).

It is interesting to point out that virtually all of the studies thus far describing an anti-inflammatory and protective effect of PECAM-1 (Table 2) have been performed in C57BL/6 mice, in which leukocyte transendothelial migration has been found to be largely PECAM-1-independent. These studies include evaluation of the effect of PECAM-1 deficiency on LPS-induced endotoxemia (Maas et al. 2005), EAE (Graesser et al. 2002), collagen-induced arthritis (Tada et al. 2003), and atherogenic diet-induced steatohepatitis (Goel et al. 2007), and atherosclerosis (Goel et al. 2008). It can be hypothesized that use of this particular strain of mice has enabled investigators to observe the anti-inflammatory effects of PECAM-1 without those effects being influenced by PECAM-1's pro-inflammatory promotion of leukocyte transmigration (Fig. 4a). In other strains of mice, wherein PECAM-1 is required for leukocyte transendothelial migration, it might be expected that the anti-inflammatory effects of PECAM-1 would be offset by its pro-inflammatory effects on leukocyte transmigration (Fig. 4b). Consequently, the ability of PECAM-1 to suppress cytokine production and maintain vascular integrity in response to inflammatory insult in C57BL/6 mice, thus lessening the severity of disease and giving the impression that PECAM-1 is mainly anti-inflammatory on the whole animal level (Fig. 4b), may not generalize to all strains of mice. It will be interesting in future studies to determine whether the predominant anti-inflammatory effect of PECAM-1, which is seen in C57BL/6 mice, is also observed when mice of other genetic strains are exposed to similar inflammatory disease models.

Isoform-specific functions of PECAM-1

It is now becoming apparent that some of the contrasting functions of PECAM-1 in inflammation might be due to differential, cell type-specific expression of alternatively spliced PECAM-1 isoforms as alternative splicing of the PECAM-1 cytoplasmic domain can affect inflammatory events, including angiogenesis, leukocyte-endothelial cell adhesion, leukocyte diapedesis, endothelial junctional stability, and cell survival in both murine and human cells (Sheibani et al. 1997; Sheibani et al. 2000; Wang et al. 2003a; Wang and Sheibani 2006; Kondo et al. 2007; Dimaio and Sheibani 2008; Bergom et al. 2008). The PECAM-1 gene consists of 16 exons, with the cytoplasmic domain being encoded from the end of exon 9 through exon 16 (Kirschbaum et al. 1994). Alternative splicing of the PECAM-1 cytoplasmic and transmembrane domains results in the production of numerous PECAM-1 isoforms, including a soluble form (Goldberger et al. 1994) and various isoforms that lack one or more cytoplasmic exons (Kirschbaum et al. 1994; Baldwin et al. 1994; Yan et al. 1995; Sheibani et al. 1997; Sheibani et al. 1999; Sheibani et al. 2000; Robson et al. 2001; Wang and Sheibani 2002; Wu and Sheibani 2003; Wang et al. 2003a; Wang et al. 2003b; Wang et al. 2004; Bergom et al. 2008; Dimaio and Sheibani 2008).

In *humans*, full length PECAM-1 is by far the predominant isoform expressed in all cells (Wang et al. 2003b), whereas PECAM-1 mRNA in *mice* tends to undergo more extensive alternative splicing with $\Delta 14,15$ (loss of exons 14 and 15) being the predominant isoform expressed in most cells (Sheibani et al. 1999). The reader is referred to a previous review that describes the production of PECAM-1 isoforms encoding differing C-terminal sequences and the tissue distribution of alternatively spliced PECAM-1 isoforms (Newman and Newman 2003).

The first biological function of PECAM-1 reported to be affected by alternative splicing was cell-cell adhesion. Thus, transfection of *mouse* PECAM-1 isoforms into mouse fibroblast L-cells was found to alter the adhesive properties of L-cell fibroblasts such that PECAM-1 participated in *heterophilic* binding interactions when exon 14 is present, but only *homophilic* binding interactions when exon 14 was absent (Yan et al. 1995). Alternatively spliced *human* PECAM-1 lacking exon 14 also modified the adhesive properties of hematopoietic cell lines (Wang et al. 2003a), leading the authors to propose that stimulus-

specific isoform switching might provide a mechanism by which PECAM-1-expressing cells, especially leukocytes, can regulate their adhesive properties.

Isoform switching of PECAM-1 also has the potential to change the signaling properties of PECAM-1-expressing cells, as only PECAM-1 isoforms that contain ITIMs encoded by exons 13 and 14 are able to efficiently recruit and activate SHP-2 (Wang and Sheibani 2006; Dimaio and Sheibani 2008; Bergom et al. 2008). This has cell-specific consequences, as expression in heterologous *Madin-Darby canine kidney (MDCK)* cells of a mouse PECAM-1 isoform containing exon 14, as opposed to one lacking exon 14, led to activation of mitogen activated protein kinases (MAPK), extracellular signal-regulated kinases (ERK), and the small GTPases Rac1 and Rap1, resulting in loss of cell-cell contacts, de-stabilization of adherens junctions, and a change in the subcellular localization of cadherins and catenins (Sheibani et al. 2000; Wang and Sheibani 2006). These effects have been proposed to explain the more migratory phenotype of PECAM-1-expressing endothelial cells during angiogenesis, as exon 14-positive PECAM-1 isoforms were found to be preferentially expressed early in vascular development, and replaced later by isoforms lacking exon 14 (Sheibani et al. 1997; Sheibani et al. 2000; Wu and Sheibani 2003); or to modulate the spatio-temporal disruption of adherens junctions downstream of PECAM-1 homophilic interactions during leukocyte transmigration (Wang and Sheibani 2006). Expression of the exon-14-containing murine PECAM-1 in an immortalized mouse brain endothelial cell line (bEND), however, had minimal effects on the activation of MAPK/ERKs and resulted in a less migratory phenotype, which was hypothesized to be due to SHP-2-mediated inhibition of signaling following recruitment to the PECAM-1 ITIMs (Dimaio and Sheibani 2008). Thus, it appears that isoform switching can change not only the adhesive properties of PECAM-1, but through loss of functional domains that bind signaling partners, it can also lead to the differential modulation of signaling pathways that can have quite divergent effects. Future studies will be required to determine which signaling pathways become activated versus suppressed by PECAM-1 in a cell-, isoform, and context-specific manner. Additionally, since any individual cell is able to express multiple PECAM-1 isoforms, it is likely that the sum biological effect of PECAM-1 on cellular signaling will reflect the relative abundance of the various isoforms that are expressed (Dimaio and Sheibani 2008).

The subcellular localization of PECAM-1 can also be influenced by isoform switching. For example, *mouse* $\Delta 15$ PECAM-1 (contains exon 14) expressed in MDCK cells does not localize to cell-cell junctions (Sheibani et al. 2000), whereas expression of this same isoform in bEND cells results in predominantly junctional localization (Dimaio and Sheibani 2008). These studies suggest that specific signaling domains of the mouse PECAM-1 cytoplasmic domain are important for determining its subcellular localization, which is especially relevant to endothelial cells where PECAM-1 tends to concentrate at the cell-cell junction (Muller et al. 1989; Newman et al. 1990; Albelda et al. 1990). Other studies have demonstrated, however, that the cytoplasmic domain of *human* PECAM-1 is not important for junctional localization, but that homophilic-mediated adhesion mediated by extracellular Ig domains determines its junctional localization (Sun et al. 2000; Bergom et al. 2008), though these studies were performed in heterologous REN cells. Though controversial, it nevertheless appears that species- and cell-specific effects modulate the subcellular localization of PECAM-1 such that homophilic binding to neighboring PECAM-1 molecules is sufficient to enable junctional localization in some cells and species, whereas additional signaling and/or trafficking mediated by cytoplasmic residues is required in others. Since endothelial cells are the relevant cell of interest for this line of investigation, studies that have used non-endothelial cell lines to ascertain the biological functions of PECAM-1 in endothelial cells might benefit by re-performing them in primary murine and human endothelial cell lines.

Concluding remarks

PECAM-1 is a multi-functional adhesion and signaling molecule that displays both pro- and anti-inflammatory effects. For pro-inflammatory effects, it has been well-established and validated in the literature that PECAM-1 is very important for the process of leukocyte transendothelial migration. PECAM-1 mediates leukocyte transmigration through adhesive interactions, the activation of integrins, and the modulation of LBRC recycling, which is important for both paracellular and transcellular leukocyte migration. The precise mechanisms through which PECAM-1 promotes integrin activation and LBRC recycling are still not completely understood. PECAM-1 has also been demonstrated to be a mechano-transducing molecule that enables endothelial cells to respond to changes in fluid shear stress (Tzima et al. 2005). This likely has implications for atherosclerotic development in areas of the vasculature exposed to disturbed shear stress as PECAM-1 can activate the pro-inflammatory transcription factor NF- κ B downstream of mechanical activation (Tzima et al. 2005). How PECAM-1 actually senses mechanical force, and how this couples to signal transduction, remains an active area of investigation.

For anti-inflammatory roles, PECAM-1 is able to dampen leukocyte activation through recruitment of inhibitory phosphatases to its cytoplasmic ITIMs (Newton-Nash and Newman 1999; Wilkinson et al. 2002; Wong et al. 2002; Rui et al. 2007). PECAM-1 also helps to dampen pro-inflammatory cytokine production and restore vascular barrier integrity through as yet poorly understood mechanisms. Future studies will need to be aimed at elucidating the specific mechanisms by which PECAM regulates these latter two processes.

On the whole organism level, the interplay of the pro- and anti-inflammatory functions of PECAM-1 has implications for both the promotion of atherosclerotic lesion development and for protection from their progression. It will be interesting in future studies to determine which of PECAM-1's biological functions predominate in both a temporal and spatial manner, and whether isoform-specific expression of PECAM-1, and/or genetic differences between mouse strains, influence these processes. Overall, a better understanding of how PECAM-1 integrates its pro- and anti-inflammatory properties during inflammatory responses will provide new insights into the biology of inflammation as well as reveal novel therapeutic targets in the treatment of both acute and chronic inflammatory disorders.

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Figure 1. The structure and function of PECAM-1

PECAM-1 is a 130 kDa type I transmembrane glycoprotein belonging to the Ig-like superfamily of adhesion molecules. The biological functions of PECAM-1 have been mapped to specific regions of the PECAM-1 molecule as shown in the figure. Residues important for mediating homophilic and heterophilic binding interactions are located within Ig-domain 1 and Ig-domains 5 & 6, respectively. Localization of PECAM-1 to membrane microdomains occurs after palmitoylation of residue Cys₅₉₅. Two ITIMs encompass residues Y₆₆₃ and Y₆₈₆ within the cytoplasmic tail and are able to serve as docking sites for cytosolic signaling molecules when the tyrosines become phosphorylated.

Figure 2. The pro-inflammatory functions of PECAM-1

PECAM-1 serves a variety of pro-inflammatory functions in both leukocytes and endothelial cells. 1. Upstream of the leukocyte adhesion cascade, PECAM-1 promotes chemokine-directed migration of leukocytes by enhancing actin polymerization and cycling. 2. The binding interaction between CD177 on neutrophils and PECAM-1 on endothelial cells is the only known heterotypic binding interaction that has been shown to be important for PECAM-1-mediated leukocyte transmigration. 3. Homotypic binding interactions between PECAM-1 on leukocytes and endothelial cells are thought to be essential for leukocyte diapedesis as blocking these interactions prevents diapedesis. 4. Downstream of homotypic binding interactions, PECAM-1 is able to activate integrins on leukocytes that are essential for adhesion to endothelial cells and passage through the basement membrane. Postulated mechanisms of PECAM-1-mediated integrin activation on leukocytes include, but are not limited to, the activation of PI3K and the GTPase Rap1. 5. During both paracellular (shown in figure) and transcellular (not shown) leukocyte transmigration, PECAM-1 is part of a surface connected membrane compartment termed the LBRC that likely serves to provide more surface area and/or unligated PECAM and other molecules to the leukocyte. 6. PECAM-1 also is able to transmit mechanical signals in endothelial cells in response to fluid forces. PECAM-1 becomes activated, and through poorly understood mechanisms, is able to transduce signals to VE-cadherin, which then activates VEGFR2. This results in the activation of integrins and the pro-inflammatory transcription factor NF- κ B, which promotes atherosclerotic lesion development.

Figure 3. PECAM-1/SHP-2 interactions dampen leukocyte activation

The PECAM-1 cytoplasmic tail contains ITIM tyrosines, which when phosphorylated, can serve as docking sites for cytosolic signaling molecules. Most times, ITIMs recruit phosphatases to counteract kinases that become activated by ITAMs. The best characterized phosphatase that is recruited by PECAM-1 is SHP-2. PECAM-1/SHP-2 interactions have been proposed to inhibit activation of B cell, T cell, mast cell, and macrophage functions as represented in the figure.

Figure 4. Proposed schematic for the interplay of the pro- and anti-inflammatory properties of PECAM-1 in different genetic strains of mice

(a) PECAM-1 is known to possess both pro- and anti-inflammatory properties as displayed in the figure, which would be proposed to largely offset each other during inflammation. (b) Most studies to date, however, have used the C57BL/6 genetic strain of mice and have described a predominant anti-inflammatory effect of PECAM-1 in models of inflammation. Loss of PECAM-1 in *most* strains of mice results in profound defects in leukocyte transmigration, however, this is not true in C57BL/6 mice, where PECAM-1 plays only a minor role in this process. As a result, the ability of PECAM-1 to suppress cytokine production and/or maintain vascular integrity in response to inflammatory insult tends to dominate in C57BL/6 mice, lessening their severity of their disease. It is hypothesized, therefore, that examining inflammatory disease models in other strains of mice, where the pro- and anti-inflammatory functions of PECAM-1 should largely offset each other, will not show predominate anti-inflammatory effects of PECAM-1.

Table 1

Pro-inflammatory functions of PECAM-1

Inflammatory Model	Results	Mouse strain	References
Neutrophil chemotaxis in Zigmund chambers toward IL-8, KC, or fMLP gradients	PECAM-1 promotes directionality, motility, spreading, and stabilizes F-actin polymerization in neutrophils	N/A	(Wu et al. 2005)
Endotoxin-induced keratitis	Antibody blockade of PECAM-1 inhibited neutrophil recruitment and prevented endotoxin-induced increases in stromal thickness and haze	BALB/c	(Khatri et al. 2002)
DSS-induced colitis	Antibody blockade of PECAM-1 reduced leukocyte transmigration and diminished disease severity	BALB/c	(Rijcken et al. 2007)
IL-1 β -induced peritonitis	PECAM-1 helps neutrophils transmigrate across the basement membrane	C57BL/6J	(Duncan et al. 1999)
Transmigration of monocytes across cultured endothelial monolayers	Antibody blockade of PECAM-1 prevents transmigration through unstimulated and cytokine-stimulated monolayers but does not prevent chemotaxis	N/A	(Muller et al. 1993)
Glycogen-induced peritonitis in rats	Antibody-blockade of PECAM-1 prevented accumulation of neutrophils into the peritoneal cavity	N/A	(Vaporciyan et al. 1993)
Glycogen-induced peritonitis in mice	Antibody-blockade of PECAM-1 prevented accumulation of neutrophils into the peritoneal cavity	AKR/J, CD2 ^{F1}	(Bogen et al. 1994)
IgG immune complex deposition in lungs in rats	Antibody-blockade of PECAM-1 blocked accumulation of neutrophils into the alveolar compartment	N/A	(Vaporciyan et al. 1993)
Human skin grafts transplanted into immunodeficient mice	Antibody-blockade of PECAM-1 blocked accumulation of neutrophils into the skin graft	Not listed	(Vaporciyan et al. 1993)
Intravital microscopy of cytokine-stimulated cremaster venules	PECAM-1 helps leukocytes transmigrate through the cell-cell junction and across the basement membrane	C57BL/6J	(Thompson et al. 2001; Dangerfield et al. 2002; Wang et al. 2005; Woodfin et al. 2009)
Thioglycollate-induced peritonitis	PECAM-1 promotes leukocyte emigration to the peritoneum	FVBn, Swiss Webster	(Schenkel et al. 2004)
Croton oil-induced topical dermatitis	PECAM-1 helps neutrophils extravasate	FVBn	(Schenkel et al. 2004)
Foreign body implant	PECAM-1 ^{+/+} mice have increased neutrophil infiltration in and around the implant compared to PECAM-1 ^{-/-} mice	C57BL/6J	(Solowiej et al. 2003)
IL-1 β - and immune complex-induced lung injury	PECAM-1 ^{+/+} mice have greater numbers of leukocytes in bronchoalveolar lavage fluid compared to PECAM-1 ^{-/-} mice	C57BL/6J	(Albelda et al. 2004)
Fluid and shear stress in the aorta	ICAM-1 staining and nuclear NF- κ B translocation is increased in PECAM-1 ^{+/+} aortas compared to PECAM-1 ^{-/-} aortas	C57BL/6J	(Tzima et al. 2005)
Atherosclerosis in LDL receptor ^{-/-} mice	PECAM-1 expression is correlated with more plaques in atherosusceptible regions of the aorta	C57BL/6J	(Harry et al. 2008; Stevens et al. 2008)

Inflammatory Model	Results	Mouse strain	References
Leukocyte transmigration across endothelial monolayers	PECAM-1 is part of the LBRC, a surface connected membrane compartment, that promotes both paracellular and transcellular migration	N/A	(Mamdouh et al. 2003; Mamdouh et al. 2008; Mamdouh et al. 2009; Dasgupta et al. 2009)
EAE	Short-term administration of chimeric soluble PECAM-1 fused to human IgG-Fc (PECAM-Fc) decreased migration of lymphocytes across brain endothelial monolayers and diminished the severity of EAE when administered at the onset of symptoms, though chronically high levels of PECAM-Fc hastened onset of EAE	SJL/J	(Reinke et al. 2007)
Collagen antibody-induced arthritis	PECAM-Fc treatment reduced inflammation and attenuated bone and cartilage destruction in symptomatic mice	DBA-1	(Dasgupta et al. 2010)

Table 2

Anti-inflammatory functions of PECAM-1

Inflammatory Model	Results	Mouse strain	References
In vitro B lymphocyte activation assays	B cells expressing PECAM-1 have lower proliferation rates in response to BCR cross-linking and decreased antibody production in response to T-independent antigens	C57BL/6J	(Wilkinson et al., 2002)
Spontaneous immune complex-mediated glomerulonephritis	Expression of PECAM-1 helps to prevent the spontaneous development of autoantibodies and immune complex-mediated glomerulonephritis	C57BL/6J	(Wilkinson et al., 2002)
In vitro T lymphocyte activation assays	Cross-linking of antibody bound PECAM-1 attenuates calcium mobilization following CD3 cross-linking	N/A	(Newton-Nash and Newman, 1999)
IgE-dependent systemic and local anaphylaxis	Expression of PECAM-1 is correlated with lower serum histamine concentrations and decreased tissue swelling and mast cell degranulation following exposure to allergic stimuli	C57BL/6J	(Wong et al., 2002)
Collagen-induced arthritis	PECAM-1 ^{+/+} mice have delayed onset of arthritis with decreased proinflammatory cytokine production and leukocyte infiltration into joints compared to PECAM-1 ^{-/-} mice	C57BL/6J	(Tada et al., 2003; Wong et al., 2005)
Experimental autoimmune encephalitis (EAE)	Expression of PECAM-1 delays the onset of EAE, decreases parenchymal inflammatory cell infiltration and promotes vascular integrity	C57BL/6J	(Graesser et al., 2002)
LPS-induced endotoxic shock	Expression of PECAM-1 is protective in LPS-induced endotoxemia and is correlated with decreased production of pro-inflammatory cytokines and increased vascular integrity	C57BL/6J	(Carrithers et al., 2005; Maas et al., 2005)
In vitro cytokine production by macrophages	Ligation and cross-linking of PECAM-1 by CD38-Fc fusion protein inhibits pro-inflammatory cytokine production in LPS-stimulated macrophages	N/A	(Rui et al., 2007)
Atherogenic-diet induced steatohepatitis	Expression of PECAM-1 delays onset and lessens severity of atherogenic diet-induced steatohepatitis by decreasing proinflammatory cytokine production and leukocyte infiltration	C57BL/6J	(Goel et al., 2007)
Atherosclerosis in LDL receptor ^{-/-} mice	Expression of PECAM-1 is correlated with decreased atherosclerotic lesion area in the total aorta with preferential protection in the aortic sinus, descending aorta, and the branching arteries of the aortic arch	C57BL/6J	(Goel et al., 2008)