Minireview

The unfolding tale of PECAM-1

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Abstract Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) is a member of the immunoglobulin (Ig) superfamily that has distinctive features of an immunoreceptor based upon its genomic structure and the presence of intrinsic immunoreceptor tyrosine inhibitory motifs (ITIMs) in its ligand binding polypeptide. This has lead to its subclassification into the Ig-ITIM superfamily. Its amino-terminal Ig-like domain of PECAM-1 is necessary for its homophilic binding, which plays an important role in cell–cell interactions. Its intracellular ITIMs serve as scaffolds for recruitment of signalling molecules including protein-tyrosine phosphatases to mediate its inhibitory co-receptor activity. Increasing evidence has implicated PECAM-1 in a plethora of biological phenomena, including modulation of integrin-mediated cell adhesion, transendothelial migration, angiogenesis, apoptosis, cell migration, negative regulation of immune cell signalling, autoimmunity, macrophage phagocytosis, IgE-mediated anaphylaxis and thrombosis. In this review, we discuss some of the new developments attributed to this molecule and its unique roles in biology.

Key words: Platelet endothelial cell adhesion molecule-1; CD31; Protein-tyrosine phosphatase; SHP-2; Immunomodulation

1. Introduction

Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) is an immunoglobulin (Ig) superfamily member expressed on the surface of platelets and leukocytes, and is also concentrated at the lateral junctions of endothelial cells. PECAM-1 is composed of six extracellular Ig-domains, a transmembrane domain and a cytoplasmic domain. Its ligands include itself (homophilic interaction involving Ig-domain 1), $\alpha_v\beta_3$ and CD38 (heterophilic interaction involving Ig-domains 1–3) [1]. Since its original cloning, much has been learned about the role of PECAM-1 in mediating various cellular interactions. PECAM-1 has been implicated in various biological functions such as leukocyte transmigration, cell migration, angiogenesis, cell signalling and cell adhesion. Biochemical and functional studies have shown that PECAM-1 contains intracytoplasmic immunoreceptor tyrosine inhibitory motifs (ITIMs), that upon phosphorylation can mediate an inhibitory function through recruitment and activation of protein-tyrosine phosphatases (PTPs), predominantly SHP-2 and to a lesser extent, SHP-1 [2–5]. Therefore, PECAM-1 is now considered a member of the Ig-ITIM superfamily, a subset of the conventional Ig-superfamily [3,4,6] (Fig. 1).

2. Expression and structure of PECAM-1

PECAM-1 has a relative molecular weight of 130-kDa and is differentially glycosylated involving N-linked and O-linked glycosylation sites. The open reading frame of PECAM-1 is composed of 16 exons, corresponding to a signal peptide, six C2-type Ig-domains, a transmembrane region and a cytoplasmic domain [7]. The localization of the PECAM-1 gene is found on the human chromosome 17q23 and the mouse chromosome 6 [8]. There are several alternatively spliced variants of PECAM-1 that are expressed in a cell-type and species-specific pattern in human, rat and mouse that arise as a result of alternative splicing of either the transmembrane or cytoplasmic domain exons. Examples include the delta-exon 14 form and the delta-13 form found in endothelial cells [9,10], a soluble form of PECAM-1 that is generated by splicing out of exon 9, which encodes the transmembrane domain [11], murine PECAM-1 delta-12,15, and delta-14,15 from developing cardiac endothelium [12] and murine PECAM-1 delta-12,14 from brain [13]. The PECAM-1 mRNA is highly expressed in lung, heart and kidney and to a lesser extent brain and liver [13]. PECAM-1 is broadly expressed in blood, immune and vascular cells. It is expressed at high density at the lateral borders of endothelial cells and at a lower density on the surface of hematopoietic and immune cells (macrophages, neutrophils, monocytes, mast cells, natural killer cells, naive T cells, naive B cells and platelets) [1]. It is not expressed on fibroblasts, epithelial cells or red blood cells.

3. Ligand interactions of PECAM-1

There is now considerable evidence that PECAM-1 mediates cellular adhesive interactions by homophilic binding of PECAM-1 on adjacent cells involving the distal N-terminal Ig-domain 1 and amino acid contact sites, D11, D33, K50, D51 and K89 on PECAM-1 [14–16]. Human–mouse chimeric studies have demonstrated species specificity for PECAM-1 homophilic interactions [14]. Apart from homophilic binding, heterophilic adhesive interactions of PECAM-1 with several ligands including integrin $\alpha_v\beta_3$, CD38, 120-kDa ligand on T cells and heparin-dependent proteoglycans have been pro-

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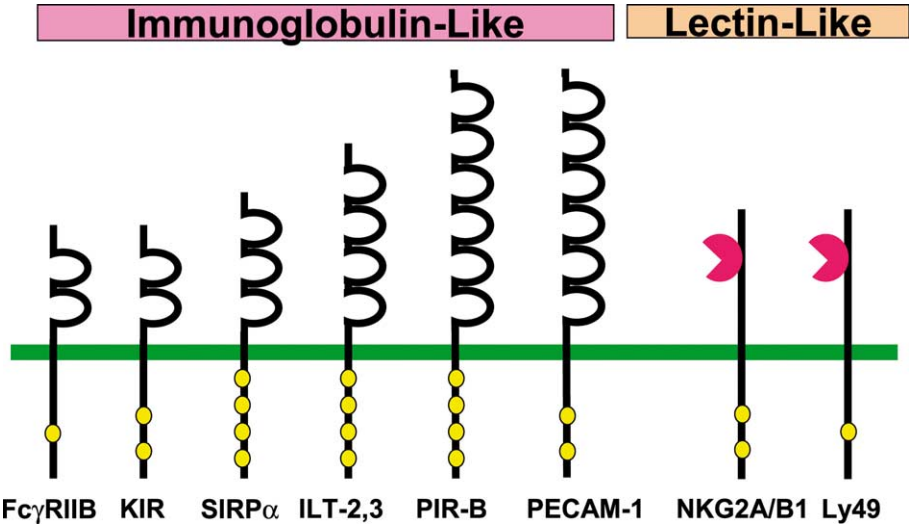

Fig. 1. Schematic representation of several ITIM-bearing receptors. Members of the Ig-ITIM superfamily include protein zero related, PZR, the low-affinity IgG receptor, FcγRIIB1, killer inhibitory receptor, KIR, the signal regulatory proteins, SIRPs, the Ig-like transcripts, ILT-2,3, paired Ig-like receptors, PIR-B, PECAM-1 and C-type lectin members, Ly49 and NKG2A/B.

posed to interact in regions encompassing Ig-domains 1–3 of PECAM-1 [17–20]. Subsequent studies have shown that cell surface glycosaminoglycans do not serve as a ligand for PECAM-1 [21]. Ligand binding specificity of PECAM-1 can be regulated by loss of tyrosine residue ITIM, Y686F from exon 14 of the cytoplasmic domain or its phosphorylation resulting in a change in ligand specificity from heterophilic to homophilic binding [22].

Recent studies have suggested that cell surface expression of PECAM-1 is further regulated by receptor shedding involving a matrix metalloproteinase-dependent mechanism and by receptor cleavage involving caspase activity [23]. PECAM-1 shedding involves the generation of a soluble 100-kDa extracellular domain form and a truncated 28-kDa fragment containing the transmembrane and cytoplasmic domain of PECAM-1 [23]. This truncated 28-kDa fragment appears to have preferential recruitment of γ-catenin and SHP-2 but not β-catenin that may enhance signalling capabilities.

4. Association of signalling molecules with PECAM-1/CD31

The human PECAM-1 cytoplasmic domain contains 12 serine, five tyrosine and five threonine residues, which could serve as docking sites for recruitment of cytosolic signalling molecules. Examination of the phosphorylation status of PECAM-1 in platelets under different conditions of stimulation has revealed that induction of serine phosphorylation of PECAM-1 is an early event in platelet activation and coincides with cytoskeletal association [2,24]. This is in contrast to the induction of tyrosine phosphorylation of PECAM-1, which is generally a later event requiring predominantly integrin $\alpha_{IIb}\beta_3$ aggregation-dependent and to a lesser extent integrin $\alpha_{IIb}\beta_3$ aggregation-independent mechanisms [2,16,25]. Platelet agonists, collagen, GPVI-selective convulxin or collagen-related peptide (CRP) and thrombin have been shown to induce tyrosine phosphorylation of PECAM-1 under conditions of platelet activation [25,26]. These studies suggested that protein-tyrosine kinase (PTK)-dependent signalling cascades such as collagen GPVI/FcR-γ chain may be functionally linked to PECAM-1-dependent signalling pathways, as tyrosine residues in ITIMs need to be phosphorylated by Src family kinase members to create docking sites for cytoplasmic *Src* homology 2 (SH2) domain-containing PTPs. Interestingly, mutagenesis studies of putative tyrosine residues converted to phenylalanine residues in the PECAM-1 ITIMs revealed that tyrosine residues, 663 and 686 account for the entire tyrosine phosphorylation of PECAM-1 and are required for PTP recruitment under conditions of pervanadate stimulation [27]. No evidence of threonine phosphorylation of PECAM-1 in platelets has been detected to date.

Several mechanisms of induction of tyrosine phosphorylation of PECAM-1 have been described. These include integrin $\alpha_{IIb}\beta_3$-mediated platelet aggregation, mechanical shear stress on endothelial cells, co-aggregation of FcεRI receptor on basophils, engagement of the T-cell receptor antigen complex (TCR) on T cells, engagement of the B-cell receptor antigen complex (BCR) on B cells, integrin engagement on extracellular matrix, cross-linking with anti-PECAM-1 antibodies, growth factor stimulation including vascular endothelial growth factor, GPVI-mediated platelet activation (via collagen or convulxin) and treatment with PTP inhibitors (vanadate and pervanadate) [2,4,25,28–32].

5. Src family kinase members

Several investigations have been undertaken to define the PTKs responsible for inducing tyrosine phosphorylation of PECAM-1. Overexpression of $p60^{c-Src}$ in endothelial cells revealed that $p60^{c-Src}$ could induce the tyrosine phosphorylation of human PECAM-1 [33]. In vitro $p60^{c-Src}$ was shown to phosphorylate a GST fusion protein containing the PECAM-1 cytoplasmic domain [14,22]. Transient overexpression of Src and Csk family members with mouse PECAM-1 in a COS cell system revealed that $p56^{lck}$, $p56^{lyn}$, $p53^{lyn}$, $p60^{c-Src}$ and $p50^{csk}$ but not Syk, Itk or Pyk2 were capable of inducing tyrosine phosphorylation of the PECAM-1 cytoplasmic domain [34]. In the context of platelets, Fyn, Lyn, Src, Yes and Hck PTKs were shown to co-immunoprecipitate with

PECAM-1 [25]. The importance of Fyn tyrosine kinase was further substantiated by showing that PECAM-1 tyrosine phosphorylation was reduced in Fyn-deficient mouse platelets [25]. In the context of $p56^{lck}$-deficient Jurkat T cells, the absence of $p56^{lck}$ resulted in loss of PECAM-1 tyrosine phosphorylation upon cross-linking the TCR complex and this could be reversed upon reconstitution of $p56^{lck}$ in these cells [5].

6. PTPs, SHP-1 and SHP-2

The first identification of PECAM-1 ITIM consensus sequences was reported by Jackson et al. [2]. Upon induction of phosphorylation of putative tyrosine residues in the ITIMs of PECAM-1, SHP-1 and SHP-2 PTPs were found to be recruited and activated. This interaction is direct and required the interaction of the SH2 domains of the PTPs with phosphorylated ITIM sequences found in exons 13 and 14 of PECAM-1 cytoplasmic domain (VQpY^{663}TEV and TVpY^{686}SEV). Importantly, these ITIM sequences classically conform to the expected consensus sequences required for PTP binding (I/VxxYxxL/V/Ix > 20a.a.I/VxYxxL/V/I). Mutagenesis studies revealed that Y663F and Y686F mutations in the PECAM-1 cytoplasmic domain led to loss of PECAM-1 tyrosine phosphorylation and recruitment of PTPs such as SHP-2 [27]. Surface plasmon resonance studies revealed that the ITIM consensus sequences bind SHP-2 at a five-fold higher affinity than SHP-1, suggesting that SHP-2 may be the preferred PTP [3]. Importantly, the association of SHP-1 and SHP-2 with tyrosine phosphorylated PECAM-1 was found to occur under physiologically relevant conditions of integrin $\alpha_{IIb}\beta_3$-mediated platelet aggregation and to a lesser degree upon platelet activation [2,3]. Subsequent studies reported PECAM-1 association with SHP-2 under conditions of FcεRI receptor aggregation in RBL-2H3 cells [29] and following mechanical stimulation of vascular endothelial cells [35]. Furthermore, transient overexpression of mouse PECAM-1 in COS cells revealed that phosphorylation of PECAM-1 by *Src* family kinases was sufficient to induce binding of both SHP-2 and SHP-1. This binding was dependent upon the integrity of the ITIM tyrosine residues, 663 and 686 [34].

7. Inositol polyphosphate 5′-phosphatase, SHIP

Phosphopeptide studies revealed that the inositol polyphosphate 5′-phosphatase, SHIP, interacted predominantly with the pY686 containing PECAM-1 cytoplasmic domain peptide [36]. Subsequent studies revealed that SHIP was capable of interacting with sequences surrounding tyrosine 686 in a non-phosphotyrosine-dependent manner [4]. Importantly, under conditions of co-aggregation of BCR complex with a chimeric receptor encompassing FcγRIIB-PECAM-1 cytoplasmic domain in DT40 B cells, PECAM-1-mediated inhibitory signalling was not dependent on the presence of SHIP [4].

Recent studies with the prototypic ITIM-bearing receptor, FcγRIIB have suggested that the consensus sequence IxpYxxL is required for SHP-1/2 binding, while xxpYxLL is the SHIP1/2 binding site in the FcγRIIB ITIM [37]. By applying these consensus sequences to the PECAM-1 ITIMs, it would appear that Y663 ITIM precisely confirms to the SHP-1/2 binding site, while Y686 ITIM conforms at Y+3 residue, but not Y−2 residue. Based upon the Y686 PECAM-1 ITIM consensus sequence, it is still not clear why SHIP would be preferentially recruited by this sequence in preference to the Y663 ITIM consensus sequence. The Y+3 residue conforms to the SHIP1/2 binding site, but not Y+2 residue. Further studies will be required to clarify this issue.

8. Phospholipase C (PLC)-γ1

Phosphopeptide studies revealed that PLC-γ1 interacted with both pY663 and pY686 containing PECAM-1 cytoplasmic domain peptides. The pY663 PECAM-1 peptide interaction appeared to be of higher affinity compared to pY686 PECAM-1 peptide interaction [36]. Examination of the PECAM-1 cytoplasmic ITIM residues demonstrated they loosely conform to the classical PLC-γ1 consensus sequences (pYLEL for N-terminal SH2 recognition) [37].

9. β- and γ-catenin

Using co-immunoprecipitation studies, it has been shown that a proportion of PECAM-1 associates with β-catenin [38]. PECAM-1 appears to be involved in controlling the localization and levels of tyrosine phosphorylated β-catenin by recruiting it to the plasma membrane and cell–cell junctions [39]. Mutation of tyrosine residues 663, 686 and 701 of PECAM-1 to phenylalanine residues did not affect β-catenin binding and co-immunoprecipitation with PECAM-1, indicating that tyrosine phosphorylation and SHP-2 recruitment were not required for β-catenin association [39]. Apart from β-catenin/PECAM-1 association, γ-catenin can also associate with PECAM-1 which is regulated by PECAM-1 serine/threonine phosphorylation but not tyrosine phosphorylation [40]. Protein kinase C (PKC)-mediated PECAM-1 phosphorylation was found to play a key role in modulating PECAM-1/γ-catenin association and localization to cell–cell junctions [40]. Whether the PECAM-1 cytoplasmic domain can directly or indirectly bind β-catenin and the relevant binding sites are not known. Further studies will be required to resolve this issue.

10. Phosphoinositide 3-kinase (PI3-kinase)

A functional association of PECAM-1 and PI3-kinase has been reported in human neutrophils [41]. Following PECAM-1 cross-linking with specific antibodies and immunoprecipitation of PECAM-1, the p85 subunit of PI3-kinase was detectable by immunoblotting in a time-dependent manner [41]. Examination of the PECAM-1 cytoplasmic domain tyrosine residues does not reveal a classical PI3-kinase consensus sequence (pYMxM). Therefore, it is possible that PI3-kinase does not directly associate with the PECAM-1 cytoplasmic domain, but is part of a multimeric signalling complex involving PECAM-1/SHP-2 with Grb2-associated binder 2 and PI3-kinase.

11. Delivery of inhibitory signalling

Based upon several studies, it would appear that co-engagement of active immunoreceptor tyrosine activation motif (ITAM)-mediated signalling (BCR, Fc receptor (FcεRI), collagen GPVI/FcR-γ chain receptor complex, platelet Fc recep-

tor (FcγRIIA) with ITIM-mediated signalling (PECAM-1) is required for delivery of 'outside-in' signalling to mediate PTP-dependent inhibitory signalling. In vitro studies have demonstrated that in order for PECAM-1 to mediate inhibitory signalling, its requires its cytoplasmic domain, intact ITIMs and specific PTPs [4,5]. The extracellular ligation requirements of PECAM-1 are less well characterized. It would appear that homophilic ligation of PECAM-1 is an important component of 'outside-in' signalling of PECAM-1 [26,42]. In vitro studies have suggested that PECAM-1 dimers, but not oligomers are efficient in supporting trans PECAM-1 homophilic interactions. Modulation of the adhesive function of integrins appears to require oligomerization of PECAM-1 within the plane of the membrane [43].

12. Cellular functions of PECAM-1

12.1. Function of PECAM-1 in B and T cells

In humans, expression of PECAM-1 is restricted to naive B cells and is down-regulated upon differentiation to memory B cells [44]. PECAM-1 is also preferentially expressed by the naive ($CD45RA^+$) $CD8^+$ T-cell subset. PECAM-1 expression is down-regulated from the majority of $CD4^+$, and about 50% of $CD8^+$ T cells as they differentiate from naive to memory T cells [45,46]. This is in contrast to the murine lymphoid environment, where PECAM-1 is expressed on 95% splenic lymphocytes and is represented across all B-2, B-1a and T-cell subsets [47]. In vitro studies using chicken DT40 B-cell lines stably transfected with FcγRIIB1-PECAM-1 wild-type (WT) and FcγRIIB1-PECAM-1 Y663,686F fusion proteins has revealed that the PECAM-1 cytoplasmic domain is capable of delivering a negative inhibitory signal that is dependent on intact ITIMs and specific PTPs, predominantly involving SHP-2 and to a lesser extent SHP-1 [4]. Co-ligation of the BCR complex with FcγRIIB1-PECAM-1 results in recruitment and activation of the PTP, SHP-2 by PECAM-1 ITIMs to inhibit downstream effector responses including Ca^{2+} flux and nuclear factor activation of transcription (NFAT) transcriptional activation [4]. Upon BCR ligation, PECAM-$1^{-/-}$ B cells exhibit enhanced calcium responses and increased B-cell proliferation [47]. This hyper-responsiveness was particularly noticeable at subthreshold concentrations of agonist used in the assay systems. PECAM-$1^{-/-}$ mice show abnormalities in B-cell development including reduced mature B-2 cells in the periphery and an overrepresentation of the B-1a pool in the peritoneal cavity.

In vivo adoptive transfer experiments using CFSE-labelled PECAM-$1^{+/+}$ and PECAM-$1^{-/-}$ lymphocytes were found to exhibit normal homing to the spleen but showed a 50% reduction of PECAM-$1^{-/-}$ B cells by day 30 after transfer compared to PECAM-$1^{+/+}$ B cells [47]. These studies suggested that the deficiency of mature recirculating B cells observed in the periphery of PECAM-$1^{-/-}$ mice may be attributed to hyper-responsive BCR signalling of PECAM-1-deficient B cells that may trigger these cells to undergo spontaneous apoptosis.

Functional evaluation of T-cell subpopulations has revealed that PECAM-1-negative $CD4^+$ T cells can provide more effective T helper cell function to recall antigens; secrete more interleukin-4 and provide more co-operation in B-cell Ig synthesis than PECAM-1-positive $CD4^+$ T cells. These differences in T-cell responsiveness in the absence of PECAM-1 could be attributed to its inhibitory receptor function. There is emerging evidence to suggest that PECAM-1 may be an important regulator of antigen-induced cell activation in the context of T lymphocytes. Induction of immune stimulation of the TCR complex on Jurkat T cells leads to tyrosine phosphorylation of PECAM-1 and recruitment of PTPs, SHP-2 and possibly SHP-1 [29]. Using $p56^{lck}$-deficient Jurkat T cells, it was shown that PECAM-1 failed to become tyrosine phosphorylated, which was restored upon reconstitution with WT $p56^{lck}$ PTK [5]. These studies indicated that in T cells, $p56^{lck}$ is the putative PTK responsible for phosphorylating PECAM-1 ITIMs. In addition, co-ligation of the ITIM-bearing PECAM-1 with TCR complex on Jurkat T cells led to a transient block in calcium mobilization [48]. Collectively, these observations suggest that PECAM-1 serves as a negative regulator of TCR-mediated signalling events.

12.2. Induction of autoimmune disease and breakdown of peripheral tolerance in PECAM-1-deficient mice

Studies on PECAM-1-deficient mice in a C57BL/6 background have shown that PECAM-1 acts as a negative regulator of immune responses in vivo and serves to prevent emergence of autoimmune disease with age [47]. These mice have increased levels of serum IgM and elevated IgG_{2a}, IgG_{2b}, IgG_3 and IgA responses to T-cell-independent antigen DNP-Ficoll, but not T-cell-dependent antigen DNP-KLH. PECAM-1-deficient mice develop anti-nuclear antibodies by 9 months of age with titers as high as 3200 and specificity for double stranded DNA. These PECAM-1-deficient mice also develop evidence of proteinuria with age and histological examination of their kidneys reveals evidence of immune-complex deposition in their glomeruli that was not observed in age- and sex-matched control mice [47]. These features are consistent with the development of autoimmune lupus-like glomerulonephritis with age.

Recognition that PECAM-1 has a functional role in regulating immunomodulation has lead to its inclusion in the list of known B-cell co-receptors including CD22, PD-1 and CD72 [47]. Biochemical and functional studies have revealed that PECAM-1 preferentially utilizes SHP-2 PTP, while CD22, PD-1 and CD72 preferentially utilize SHP-1 PTP to modulate BCR-mediated signalling. Therefore, it is likely that these PTPs may regulate different signalling pathways and regulate B-cell responsiveness in different contexts. Further studies will be required to examine whether different B-cell co-receptors utilize similar or different pathways to modulate BCR-mediated signalling.

In an inducible mouse model of autoimmune encephalomyelitis (EAE) that resembles the human autoimmune disease of multiple sclerosis, PECAM-1-deficient mice demonstrated an early onset of clinical symptoms of EAE and increased immune cell trafficking into the central nervous system during early stages of the disease [49].

12.3. Function of PECAM-1 in platelets

Even though PECAM-1 was originally identified to be expressed in platelets over 10 years ago, its functional importance has been unclear. As no individuals have been reported that have qualitative or quantitative defects in PECAM-1, the availability of PECAM-1-deficient mice has provided the opportunity to directly test its involvement in hemostasis and thrombogenesis. Initial assessment of PECAM-1-deficient

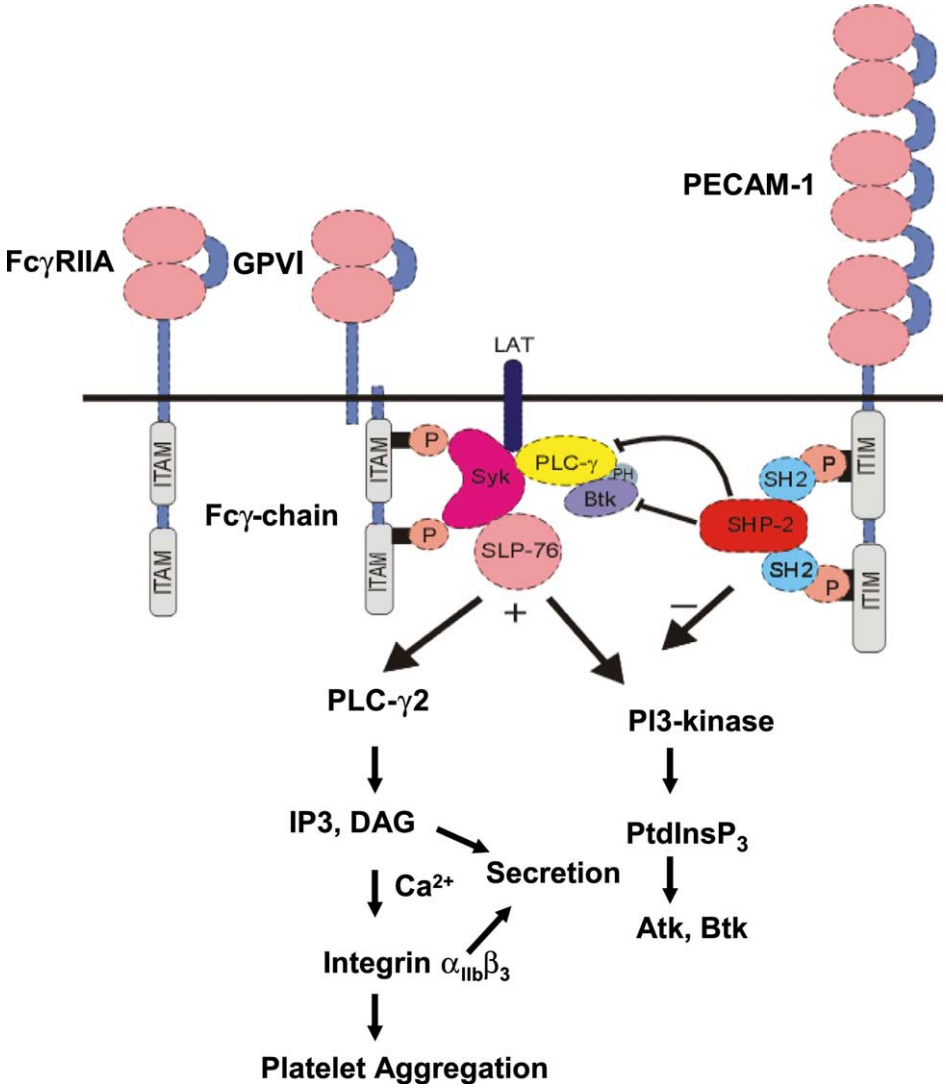

Fig. 2. Schematic representation of ITAM/ITIM-bearing receptor pathways in human platelets. Upon clustering of GPVI or FcγRIIA, the ITAMs are phosphorylated to allow recruitment of Syk, which then phosphorylates PLC-γ2. GPVI and FcγRIIA can also recruit PI3-kinase, which upon activation promotes rapid accumulation of PtdIns(3,4,5)P_3 and downstream phosphorylation of the serine/threonine Akt or Bruton's tyrosine kinase, Btk. PtdInsP_3 allows binding of PLC-γ2 to the membrane/cytoskeleton, enabling activation of the enzyme and generation of secondary messengers including inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG). These signalling events lead to calcium mobilization and activation of PKC that promote platelet secretion and engagement of integrin $\alpha_{IIb}\beta_3$ resulting in platelet aggregation. A negative feedback signal is created by the co-engagement of PECAM-1 with recruitment and activation of PTPs including SHP-2 delivering negative inhibitory signalling to phosphorylate many of the components of the FcγRIIA and GPVI/FcR-γ chain ITAM-associated signalling pathways.

mice demonstrated that megakaryocyte and platelet production was normal with no abnormality detected in ADP-induced platelet aggregation [50]. PECAM-1-deficient mice display a prolonged bleeding time, which has been attributed to a vascular defect rather than a platelet defect, as irradiation of PECAM-1-deficient mice and reconstitution of the hematopoietic cell compartment containing PECAM-1-positive platelets did not correct the bleeding time [51]. As PECAM-1 is first Ig-ITIM-bearing receptor to be characterized in platelets, it is likely that it would negatively modulate ITAM-bearing cascades including collagen receptor GPVI/FcR-γ chain and FcγRIIA-mediated signalling. Under conditions of static platelet adhesion, PECAM-$1^{-/-}$ platelets showed enhanced platelet adhesion on immobilized collagen but not fibrinogen or bovine vWF matrix compared to WT platelets, indicating a selectivity for collagen. PECAM-1-deficient platelets displayed enhanced platelet aggregation and secretion responses on stimulation with varying doses of collagen and CRP, although the responses to ADP and thrombin over a wide dose range were unaffected [26,52]. Using both human and mouse models, PECAM-1 was demonstrated to act as a physiological negative regulator of platelet–collagen interactions that may function to negatively limit growth of platelet thrombi on collagen surfaces [26]. Specifically, under conditions of flow, human thrombus formation on immobilized collagen was reduced in a dose-dependent manner by human PECAM-1-Ig chimera but not control IgG. Platelets derived from PECAM-1-deficient mice formed larger thrombi when perfused over a collagen matrix under flow at a shear rate of 1800 s^{-1} compared to WT mice [26]. These studies demonstrate that PECAM-1 has the ability to negatively modulate the prothrombotic platelet–collagen interactions suggesting that it serves a

pivotal role in preventing platelet thrombus formation. Our recent studies have highlighted that PECAM-1 also serves as a potent negative modulator of FcγRIIA-dependent platelet responses including HITS-mediated platelet aggregation (Thai, L.M. and Jackson, D.E., unpublished observations). Both these studies support a role for PECAM-1 in modulating two important platelet active receptor pathways involving collagen GPVI/FcR-γ chain and FcγRIIA (Fig. 2).

Recent studies have raised the possibility that PECAM-1 may negatively regulate non-ITAM-associated G-protein coupled receptor signalling in platelets. In these experiments, cross-linking of PECAM-1 inhibited thrombin and ADP-mediated aggregation and secretion of platelets but only at low dose concentrations of thrombin and ADP [51]. These findings have not been confirmed in studies of PECAM-1-deficient platelets when examining a wide range of concentrations of thrombin and ADP [26,50,53]. The reason for this discrepancy is not clear and will require further investigation.

12.4. Function of PECAM-1 in endothelial cells

PECAM-1 is highly expressed in the vasculature with approximately one million copies of PECAM-1 reported on the surface of endothelial cells [54]. Given its abundant expression in endothelial cells, PECAM-1 has been demonstrated to be involved in the initial formation and stabilization of cell–cell contacts at lateral junctions of endothelial cells, the maintenance of a vascular permeability barrier, modulation of cell migration, transendothelial migration of monocytes and neutrophils and formation of new blood vessels in angiogenesis [55–61]. Recent studies using cultured PECAM-1-deficient endothelial cells have shown enhanced permeability changes in response to histamine compared to PECAM-1-reconstituted endothelial cells [49]. These studies provided confirmatory evidence that PECAM-1 has an important function in maintenance of a vascular permeability barrier.

Overexpression of a mutant form, Y686F PECAM-1 or a truncated PECAM-1 construct devoid of the extracellular domain, in BAECs led to increased cell migration rates. These studies highlighted that cytoplasmic domain ITIMs and the extracellular domain of PECAM-1 may play an important role in 'inside-out' and 'outside-in' signalling events to modulate endothelial cell migration [62]. In rat and murine models of angiogenesis involving endothelial cells suspended in a three-dimensional gel composed of type I collagen, polyclonal and monoclonal antibodies directed to PECAM-1 were found to block in vitro tube formation [61]. In a similar model, endothelial cells that were incubated with anti-PECAM-1 antibodies showed reduced cell elongation and migration, but no inhibition of the process of vacuole formation was observed [63]. Thus, these studies highlighted that PECAM-1 may play a role in the invasion, migration and/or extension of pseudopods through a type I collagen matrix.

Maintenance of the lateral localization of PECAM-1 at the junctions of endothelial cells requires cell contact. Without cell contact, PECAM-1 remains diffusely distributed within the plane of the membrane of endothelial cells until it encounters its homophilic ligand, PECAM-1 on an adjacent cell [56]. Recent studies with chimeric constructs of human PECAM-1 have revealed that homophilic binding of PECAM-1 on adjacent cells and positively charged sequences (^{599}RKAKAK^{604}) juxtamembrane to the transmembrane domain of PECAM-1 are important for its localization to cell–cell borders [64]. These highly charged amino acids may provide important docking sites of the recruitment of cytosolic signalling molecules and/or cytoskeletal proteins to support PECAM-1 localization and stabilization at cell–cell borders.

12.5. Function of PECAM-1 in leukocytes

An involvement of PECAM-1 has been implicated in neutrophil recruitment in vivo, neutrophil and monocyte chemotaxis and transendothelial migration of monocytes and neutrophils in vitro [60,65,66]. Anti-domain 1 PECAM-1 monoclonal antibodies and soluble PECAM-IgG chimeric molecules have been shown to inhibit transmigration of leukocytes through endothelium and in vivo neutrophil recruitment [60,65,67–69].

In the process of macrophage phagocytosis, when a healthy cell encounters a macrophage, signalling through bound PECAM-1 receptors leads to the cells being repelled and disruption of cell adhesion. This is in contrast to dying cells undergoing apoptosis, where signalling through PECAM-1 is somehow disrupted resulting in the cells not being repelled and the balance being shifted towards engagement of macrophage engulfment receptors [42,70]. Recent studies implicate PECAM-1 as a potent suppressor of Bax mitochondrial-mediated apoptosis and suggests an unrecognized function for PECAM-1 in the biology of blood and vascular cells [71].

12.6. Function of PECAM-1 in mast cells

In RBL-2H3 cells, aggregation of the high-affinity Fc receptor for IgE, FcεRI with anti-receptor antibodies induced the tyrosine phosphorylation of PECAM-1 and recruitment of SHP-2 PTP. This receptor-induced tyrosine phosphorylation event was independent of Ca^{2+} flux, activation of PKC and of cell adhesion [29,30]. Recent studies have suggested that PECAM-1 may play an important role in modulation of mast cell function. PECAM-1$^{-/-}$ mice exhibited enhanced IgE-mediated systemic anaphylaxis and showed increased sensitivity to local cutaneous IgE-dependent anaphylaxis compared to PECAM-1$^{+/+}$ mice [72]. PECAM-1$^{-/-}$ bone marrow-derived mast cells (BMMCs) showed enhanced serotonin release following IgE stimulation and cross-linking with DNP-BSA in vitro compared to WT BMMCs [72]. These functional studies suggested that PECAM-1 acts as a counter-regulator in allergic disease susceptibility and severity.

13. Concluding remarks

Based upon recent work we now consider PECAM-1 as a member of the Ig-ITIM superfamily that is emerging as an important receptor that has a repertoire of diverse functions ranging from modulation of apoptosis, immune responses, integrin-dependent adhesive events, angiogenesis, transendothelial migration, macrophage phagocytosis, platelet responsiveness, mast cell degranulation and cell motility. Taken together, PECAM-1 has a pivotal role in modulating cell signalling pathways that are essential in prevention of autoimmunity, thrombosis, allergy and cancer. Further studies will help to unravel the involvement of PECAM-1 in normal and pathological processes.

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References

[1] Newman, P.J. (1997) J. Clin. Invest. 100, S25–S29.
[2] Jackson, D.E., Ward, C.M., Wang, R. and Newman, P.J. (1997a) J. Biol. Chem. 272, 6986–6993.
[3] Hua, C., Gamble, J., Vadas, M. and Jackson, D.E. (1998) J. Biol. Chem. 273, 28332–28340.
[4] Henshall, T.L., Jones, K.L., Wilkinson, R. and Jackson, D.E. (2001) J. Immunol. 166, 3098–3106.
[5] Newman, D.K., Hamilton, C. and Newman, P.J. (2001) Blood 97, 2351–2357.
[6] Newman, P.J. (1999) J. Clin. Invest. 103, 5–9.
[7] Newman, P.J., Berndt, M.C., Gorski, J., White II, G.C., Lyman, S., Paddock, S. and Muller, W.A. (1990) Science 247, 1157–1264.
[8] Gumina, R.J., Kirschbaum, N.E., Rao, P.N., VanTuinen, P. and Newman, P.J. (1996) Genomics 34, 229–232.
[9] Kirschbaum, N.E., Gumina, R.J. and Newman, P.J. (1994) Blood 84, 4028–4037.
[10] Osawa, M., Masuda, M., Harada, N., Lopes, R.B. and Fujiwara, K. (1997) Eur. J. Cell. Biol. 72, 229–237.
[11] Goldberger, A., Middleton, K.A., Oliver, J.A., Paddock, C., Yan, H.C., DeLisser, H.M., Albelda, S.M. and Newman, P.J. (1994) J. Biol. Chem. 269, 17183–17191.
[12] Baldwin, H.S., Shen, H.M., Yan, H.C., DeLisser, H.M., Chung, A., Mickanin, C., Trask, T., Kirschbaum, N.E., Newman, P.J., Albelda, S.M. and Buck, C.A. (1994) Development 120, 2539–2553.
[13] Sheibani, N., Sorenson, C.M. and Frazier, W.A. (1999) Dev. Dyn. 214, 44–54.
[14] Sun, Q.H., DeLisser, L.M., Zukowski, M.M., Paddock, C., Albelda, S.M. and Newman, P.J. (1996) J. Biol. Chem. 271, 11090–11098.
[15] Sun, J., Williams, J., Yan, H.-C., Amin, K.M., Albelda, S.M. and DeLisser, H.M. (1996) J. Biol. Chem. 271, 18561–18570.
[16] Newton, J.P., Buckley, C.D., Jones, E.Y. and Simmons, D.L. (1996) J. Biol. Chem. 272, 20555–20563.
[17] Piali, L., Hammel, P., Uherek, C., Bachmann, F., Gisler, R.H., Dunon, D. and Imhof, B.A. (1995) J. Cell. Biol. 130, 451–460.
[18] Deaglio, S., Morra, M., Mallone, R., Ausiello, C.M., Prager, E., Garbarino, G., Dianzani, U., Stockinger, H. and Malavasi, F. (1998) J. Immunol. 160, 395–402.
[19] Prager, E., Sunder-Plassmann, R., Hansmann, C., Kock, C., Holter, W., Knapp, W. and Stockinger, W. (1996) J. Exp. Med. 184, 41–50.
[20] DeLisser, H.M., Yan, H.C., Newman, P.J., Muller, W.A., Buck, C.A. and Albelda, S.M. (1993) J. Biol. Chem. 268, 16037–16046.
[21] Sun, Q.H., Paddock, C., Visentin, G.P., Zukowski, M.M., Muller, W.A. and Newman, P.J. (1998) J. Biol. Chem. 273, 11483–11490.
[22] Famiglietti, J., Sun, J., DeLisser, H.M. and Albelda, S.M. (1997) J. Cell. Biol. 138, 1425–1435.
[23] Ilan, N., Mohsenin, A., Cheung, L. and Madri, J.A. (2001) FASEB J. 15, 362–372.
[24] Newman, P.J., Hillery, C.A., Albrecht, R., Parise, L.V., Berndt, M.C., Mazurov, A.V., Dunlop, L.C., Zhang, J. and Rittenhouse, S.E. (1992) J. Cell. Biol. 119, 239–246.
[25] Cicmil, M., Thomas, J.M., Sage, T., Barry, F.A., Leduc, M., Bon, C. and Gibbons, J.M. (2000) J. Biol. Chem. 275, 27339–27347.
[26] Jones, K.L., Hughan, S.C., Dopheide, S.M., Farndale, R.W., Jackson, S.P. and Jackson, D.E. (2001) Blood 98, 1456–1463.
[27] Jackson, D.E., Kupcho, K.R. and Newman, P.J. (1997b) J. Biol. Chem. 272, 24868–24875.
[28] Osawa, M., Masuda, M., Harada, N., Lopes, R.B. and Fujiwara, K. (1997) Eur. J. Cell. Biol. 72, 229–237.
[29] Sagawa, K., Kimura, T., Swieter, M. and Siraganian, R.P. (1997b) J. Biol. Chem. 272, 31086–31091.
[30] Sagawa, K., Swaim, W., Zhang, J., Unsworth, E. and Siraganian, R.P. (1997a) J. Biol. Chem. 272, 13412–13418.
[31] Varon, D., Jackson, D.E., Shenkman, B., Dardik, R., Tamarin, I., Savion, N. and Newman, P.J. (1998) Blood 91, 500–507.
[32] Esser, S., Lampugnani, M.G., Corada, M., Dejana, E. and Risau, W. (1998) J. Cell. Sci. 111, 1853–1865.
[33] Lu, T.T., Barreuther, M., Davis, S. and Madri, J.A. (1997) J. Biol. Chem. 272, 14442–14446.
[34] Cao, M.Y., Huber, M., Beauchemin, N., Famiglietti, J., Albelda, S.M. and Veillette, A. (1998) J. Biol. Chem. 273, 15765–15772.
[35] Masuda, M., Osawa, M., Shigematsu, H., Harada, N. and Fujiwara, K. (1998) FEBS Lett. 408, 331–336.
[36] Pumphrey, N.J., Taylor, V., Freeman, S., Douglas, M.R., Bradfield, P.F., Young, S.P., Lord, J.M., Wakelam, M.J.O., Bird, I.N., Salmon, M. and Buckley, C.D. (1998) FEBS Lett. 450, 77–83.
[37] Bruhns, P., Vely, F., Malbec, O., Fridman, W.H., Vivier, E. and Daeron, M. (2000) J. Biol. Chem. 275, 37357–37364.
[38] Matsumura, T., Wolff, K. and Petzelbauer, P. (1997) J. Immunol. 158, 3408–3416.
[39] Ilan, N., Mahooti, S., Rimm, D.L. and Madri, J.A. (1999) J. Cell. Sci. 112, 3005–3014.
[40] Ilan, N., Cheung, L., Pinter, E. and Madri, J.A. (2000) J. Biol. Chem. 275, 21435–21443.
[41] Pellegatta, F., Chierchia, S.L. and Zocchi, M.R. (1998) J. Biol. Chem. 273, 27768–27771.
[42] Brown, S., Heinisch, I., Ross, E., Shaw, K., Buckley, C.D. and Savill, J. (2002) Nature 418, 200–203.
[43] Zhao, T. and Newman, P.J. (2001) J. Cell. Biol. 152, 65–73.
[44] Jackson, D.E., Gully, L.M., Henshall, T.L., Mardell, C.E. and Macardle, P.J. (2000) Tissue Antigens 56, 105–116.
[45] Ashman, L.K. and Aylett, G.W. (1991) Tissue Antigens 38, 208–212.
[46] Tanaka, Y., Albelda, S.M., Horgan, K.J., van Seventer, G.A., Shimizu, Y., Newman, W., Hallam, J., Newman, P.J., Buck, C.A. and Shaw, S. (1992) J. Exp. Med. 176, 245–253.
[47] Wilkinson, R., Lyons, A.B., Roberts, D., Wong, M.X., Bartley, P.A. and Jackson, D.E. (2002) Blood 100, 184–193.
[48] Newton-Nash, D.K. and Newman, P.J. (1999) J. Immunol. 163, 682–688.
[49] Graesser, D., Solowiej, A., Bruckner, M., Osterweil, E., Juedes, A., Davis, S., Ruddle, N.H., Engelhardt, B. and Madri, J.A. (2002) J. Clin. Invest. 109, 383–392.
[50] Duncan, G.S., Andrew, D.P., Takimoto, H., Kaufman, S.A., Yoshida, H., Spellberg, J., Lius de la Pompa, J., Elia, A., Wakeham, A., Karan-Tamir, B., Muller, W.A., Senaldi, G., Zukowki, M.M. and Mak, T.W. (1999) J. Immunol. 162, 3022–3030.
[51] Mahooti, S., Graesser, D., Patil, S., Newman, P.J., Duncan, G.S., Mak, T. and Madri, J.A. (2000) Am. J. Pathol. 157, 75–81.
[52] Patil, S., Newman, D.K. and Newman, P.J. (2001) Blood 97, 1727–1732.
[53] Cicmil, M., Thomas, J.M., Leduc, M., Bon, C. and Gibbins, J.M. (2002) Blood 99, 137–144.
[54] Newman, P.J. (1994) Ann. N.Y. Acad. Sci. 714, 165–174.
[55] Newman, P.J., Berndt, M.C., Gorski, J., White 2nd, G.C., Lyman, S., Paddock, C. and Muller, W.A. (1990) Science 247, 1219–1222.
[56] Albelda, S.M., Oliver, P.D., Romer, L.H. and Buck, C.A. (1990) J. Cell. Biol. 110, 1227–1237.
[57] Albelda, S.M., Muller, W.A., Buck, C.A. and Newman, P.J. (1991) J. Cell. Biol. 114, 1059–1068.
[58] Ferrero, E., Ferrero, M.E., Pardi, R. and Zocchi, M.R. (1991) FEBS Lett. 374, 323–326.
[59] Schimmenti, L.A., Yan, H.C., Madri, J.A. and Albelda, S.M. (1992) J. Cell. Physiol. 153, 417–428.
[60] Muller, W.A., Weigl, S.A., Deng, X. and Phillips, D.M. (1993) J. Exp. Med. 178, 449–460.
[61] DeLisser, H.M., Christofidou-Solomidou, M., Strieter, R.M., Burdick, M.D., Robinson, C.S., Wexler, R.S., Kerr, J.S., Garlanda, C., Merwin, J.R., Madri, J.A. and Albelda, S.M. (1997) Am. J. Pathol. 151, 671–677.
[62] Kim, C.S., Wang, T. and Madri, J.A. (1998) Lab. Invest. 78, 583–590.
[63] Yang, S., Graham, J., Kahn, J.W., Schwartz, E.A. and Gerritsen, M.E. (1999) Am. J. Pathol. 155, 887–895.
[64] Sun, J., Paddock, C., Shubert, J., Zhang, H.-B., Amin, K., Newman, P.J. and Albelda, S.M. (2000) J. Cell. Sci. 113, 1459–1469.
[65] Vaporciyan, A.A., DeLisser, H.M., Yan, H.C., Mendiguren, I.I., Thom, S.R., Jones, M.L., Ward, P.A. and Albelda, S.M. (1993) Science 262, 1580–1582.

[66] Bogen, S., Pak, J., Garifallou, M., Deng, X. and Muller, W.A. (1994) J. Exp. Med. 179, 1059–1064.
[67] Liao, F., Ali, J., Green, T. and Muller, W.A. (1997) J. Exp. Med. 185, 1349–1357.
[68] Liao, F., Schenkel, A.R. and Muller, W.A. (1999) J. Immunol. 163, 5640–5648.
[69] Nakada, M.T., Amin, K., Christofidou-Solomidou, M., O'Brien, C.D., Sun, J., Gurubhagavatula, I., Heavner, G.A., Taylor, A.H., Paddock, C., Sun, Q.-H., Zehnder, Z.L., Newman, P.J., Albelda, S.M. and DeLisser, H.M. (2000) J. Immunol. 164, 452–462.
[70] Chimini, G. (2002) Nature 418, 139–140.
[71] Gao, C., Sun, W., Matsuyama, S. and Newman, P.J. (2001) Blood 98, 796a.
[72] Wong, M.X., Roberts, D., Bartley, P.A. and Jackson, D.E. (2002) J. Immunol. 168, 6455–6462.