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Platelet endothelial cell adhesion molecule-1 (PECAM-1, or CD31) is a 130-kDa member of the immunoglobulin (Ig) superfamily that is expressed on the surface of circulating platelets, monocytes, neutrophils, and particular T-cell subsets. It is also a major constituent of the endothelial cell intercellular junction, where up to an estimated one million molecules are concentrated. Because of this cellular expression pattern, PECAM-1 is implicated in several functions, including transendothelial migration of leukocytes, angiogenesis, and integrin activation (for a review of the biology of PECAM-1, see ref. 1). When the cDNA sequence for PECAM-1 was determined more than eight years ago (2), it was originally assigned to the growing family of Ig-like cell adhesion molecules (Ig-CAMs). This classification was the result of the sequence similarity of PECAM-1's extracellular Ig domains to those of other Ig-CAMs characterized at that time, including carcinoembryonic antigen (CEA), intercellular adhesion molecule-1 (ICAM-1), and neural cell adhesion molecule (NCAM). Recent observations however, suggest that we may have been following the wrong half of the molecule and that PECAM-1 may not be a member of the Ig-CAM family after all.

Ig-superfamily members have been known for some time to mediate cell adhesion (e.g., NCAM, ICAM-1, and vascular cell adhesion molecule-1 [VCAM-1]) or antigen recognition (e.g., immunoglobulins, T-cell receptors, and MHC molecules). However, a subgroup comprising 30 members characterized by the presence of one or more immunoreceptor tyrosine-based *inhibitory* motifs (ITIMs) within their cytoplasmic domain has recently emerged (3, 4). Currently recognized members of the Ig-ITIM family are listed in Table 1. They include members of the killer inhibitory receptor (KIR) family, the murine paired Ig-like receptor-braking protein (PIR-B), a low-affinity receptor for IgG (FcγRIIb), at least two members of the Ig-like transcript (ILT) family, a transmembrane domain-containing member of the CEA family known as biliary glycoprotein-1 (BGP-1), cytotoxic T lymphocyte-associated protein-4 (CTLA-4), and the B cell-specific antigen, CD22. When members of the Ig-ITIM family are appropriately engaged, they become phosphorylated on distinct tyrosine residues located within their cytoplasmic ITIM resulting in the creation of specific docking sites for Src-homology 2 (SH2) domain-containing intracellular lipid- and protein-tyrosine phosphatases, such as SHIP, SHP-1, or SHP-2.

Table 1
ITIM-containing inhibitory receptors

Name	Mass (kDa)	Chromosome	No. Ig domains	Phosphatase bound	Ligands	Cellular distribution
KIR (CD158a)	58/70	19	2-3	SHP-1 & 2	MHC class I	NK, subpop of T cells
muPIR-B (p91)	130/110	*7	6	SHP-1 & 2	?	B, PMN, Mo macro, mast
FcγRIIb (CD32)	40	1	2	SHIP	IgG (low affinity)	B-cells, mast cells
ILT2	68	19	4	?	?	B-cells
ILT3	59	19	2	SHP-1	?	Macrophage, Mo, dendritic
BGP-1 (CD66a)	160	19	4	SHP-1	BGP-1	Epi, EC, B, PMN, macrophage
SIRPs	110	?	3	SHP-2 & 1	?	ubiquitous
CTLA-4 (CD 152)	25	2	1	SHP-2	B7.1, B7.2	Activated T-cells
CD22	140	19	7	SHP-1	α2,6 sialic acid	B-cells
PECAM-1 (CD31)	130	17	6	SHP-2 & 1	PECAM-1	Plt, EC, Mo, PMN, T, B

KIR actually represents a highly polymorphic multigene family of receptors capable of inhibiting NK and T-cell activation, and contains either two or three extracellular Ig-homology domains (50). Similarly, SIRPs (also known as SHPS-1 and BIT) represent a family having at least 15 members (51), and BGP-1 is one of more than 29 members of the carcinoembryonic antigen family (52). FcγRIIb (CD32 containing a cytoplasmic ITIM) is the only Fc receptor on B cells, whereas its counterpart, FcγRIIIa (CD32 containing a cytoplasmic ITAM), is the only Fc receptor on platelets. The murine PIR consist of two closely related proteins, PIR-Activating and PIR-Braking, having 92% identity in their extracellular domains, while their transmembrane and cytoplasmic domains are distinct. Thus, PIR-A has a short cytoplasmic domain containing a charged amino acid thought to mediate a polar interaction with an ITAM-containing subunit, while PIR-B contains four potential ITIMs within its long cytoplasmic domain (26). The PIR genes have been localized to the proximal end of murine chromosome 7 in a region that is syntenic to human chromosome 19q13 (53), where, as shown above, the genes for several other Ig-ITIM family members have been localized. Note that PECAM-1 is the only Ig-ITIM family member to have been identified on platelets to date and is also abundantly expressed on endothelial cells, where up to one million molecules concentrate at intracellular junctions shortly after cell-cell contact. B, B lymphocytes; BGP-1, biliary glycoprotein-1; EC, endothelial cells; Epi, epithelial cells of the GI tract; Ig, immunoglobulin; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; KIR, killer inhibitory receptor; Mo, monocytes; NK, natural killer cells; PECAM-1, platelet endothelial cell adhesion molecule-1; PIR, paired Ig-like receptors; Plt, platelets; PMN, polymorphonuclear leukocytes; SIRPs, signal regulatory proteins; T, T lymphocytes.

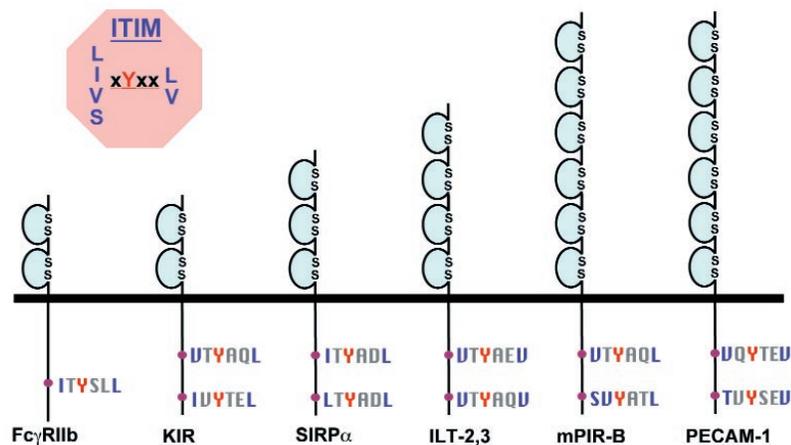


Figure 1

Schematic diagram of selected Ig-superfamily members bearing one or more ITIMs (adapted from ref. 48). See Table 2 for a complete list of Ig-ITIM family members that bind SHP-1, SHP-2, or both, as well as the detailed amino acid sequence separating the paired ITIMs. *Ig*, immunoglobulin; *ILT*, Ig-like transcript; *ITIM*, immunoreceptor tyrosine-based inhibitory motif; *KIR*, killer inhibitory receptor; *PECAM-1*, platelet endothelial cell adhesion molecule-1; *PIR-B*, paired Ig-like receptor-braking protein; *SIRP*, signal regulatory protein.

These catalytic enzymes, once localized to their cytoplasmic anchors and activated, are then able to effect a wide range of cellular events, most notably inhibition of tyrosine kinase-mediated signaling, proliferation, and cellular activation.

ITIMs are identifiable by the consensus sequence L/I/V/S-x-Y-x-x-L/V and have been found to exist alone or in pairs within the cytoplasmic domain of an increasingly recognized number of inhibitory receptors. For example, the inhibitory Fcγ receptor, FcγRIIb, harbors a single ITIM within its cytoplasmic domain and signals predominantly through SHIP, an inositol phosphatase that binds to the ITIM via its single amino-terminal SH2 domain. On the other hand, Ig-ITIM family members that bind SHP-1 and SHP-2 most commonly contain two or more ITIMs separated by at least 20 residues (50 Å) each — a feature that no doubt contributes specificity to their recruitment and activation of these tandem SH2 domain-containing protein-tyrosine phosphatases (5). The distance separating ITIMs is in sharp contrast to the much shorter spacing between the bisphosphotyrosyl sequences present in immunoreceptor tyrosine-based activation motifs (ITAMs; consensus = Y-x-x-L-x₆₋₈-Y-x-x-L), which are located within the cytoplasmic domains of stimulatory receptors such as T-cell receptor ζ chain, the Fc receptor γ chain (a 14-kDa signaling subunit that is associated with FcγRI, FcγRIII, FcεRI and platelet GPVI), all three CD3 subunits (ε, γ, and δ), the Igα/Igβ dimer that is associated with the μ chain of the B-cell receptor, and FcγRIIa (for recent reviews of the biology of ITAMs, see refs. 6 and 7). As a consequence of the close proximity of their phosphotyrosine residues, ITAMs appear to have a much higher affinity for the SH2 domains of protein-tyrosine kinases like ZAP-70, Syk, and phosphatidylinositol-3-kinase (8) than they do for the more widely spaced SH2 domains of protein-tyrosine phosphatases (5). This fact has only been appreciated of late, however, resulting in

the inadvertent assignment of a number of proteins containing canonical ITIMs to the ITAM family of stimulatory receptors (9–11).

PECAM-1 appears to be one such example. Several years ago, Modderman *et al.* (12) found that PECAM-1 could become phosphorylated on tyrosine residues after treatment of platelets with pervanadate, a protein-tyrosine phosphatase inhibitor. Subsequently, Jackson *et al.* (13, 14) showed that two specific tyrosine residues, Y₆₆₃ and Y₆₈₆, located within the 118-amino acid PECAM-1 cytoplasmic domain, formed a specific docking site for the protein-tyrosine phosphatase, SHP-2, and that this signaling molecule bound avidly to PECAM-1 after platelet aggregation. This has since been confirmed by several investigators (15, 16), and the number of cellular activation events that can lead to PECAM-1 tyrosine phosphorylation and SHP-2 binding has been expanded to include shear (17) or oxidative (18) stress, osmotic shock (17), exposure to lysophosphatidyl choline (19), and monoclonal antibody-induced cross-linking of the T-cell receptor (20), the Fcε receptor (20), or PECAM-1 itself (21). Thus, PECAM-1 appears to fulfill one of the criteria established for inclusion in the Ig-ITIM family (3): it recruits one or more SH2 domain-containing phosphatases after phosphorylation. In addition, the paired ITIMs within its cytoplasmic domain are remarkably similar to those present in other well-established members of the Ig-ITIM family (Fig. 1 and Table 2). Taken together, it would appear that PECAM-1 is, in fact, a member of the Ig-ITIM family, and may not necessarily have cell adhesion as its primary function after all. Rather, like other members of this family, PECAM-1 may function as an inhibitory receptor, serving to moderate or attenuate tyrosine kinase-mediated signaling pathways, or to set thresholds for cellular activation in the vascular cells that express it.

Further support for inclusion of PECAM-1 within the Ig-ITIM, rather than the Ig-CAM, family can be found by comparing the genomic organization of PECAM-1 with

genes that encode two representative Ig-CAMs (ICAM-1 and VCAM-1) and two Ig-ITIM family members (CD22 and PIR-B). As shown in Fig. 2, the Ig-homology domains that comprise the extracellular region of each of these genes are encoded by individual exons — a feature common among members of the Ig superfamily (22); one notable exception is NCAM (23). However, whereas the transmembrane domain, the cytoplasmic domain, and the 3' untranslated region are normally encoded by a single exon for Ig-CAMs, these domains are often split into six or more small exons in genes encoding members of the Ig-ITIM family. Notably, the ITIMs themselves are commonly encoded by separate exons (coincidentally, exons 13 and 14 for both PECAM-1 and CD22), providing the potential to generate alternatively spliced isoforms that differ in both structure and function (24, 25). Moreover, the signal peptide in several Ig-ITIM family members, including KIR, PIR-B, and CD22 (26), is split between two exons, with the second exon being a mini-exon 25–40 bp in length. PECAM-1 shares this unusual genomic organization.

Reexamination of the literature provides still further evidence that PECAM-1 functions as an inhibitory receptor in vascular cells, although much of this evidence is circumstantial. First, Schimmenti *et al.* (27) found more than eight years ago that transfecting full-length PECAM-1 into NIH/3T3 cells causes them to migrate more slowly — a feature that was at that time attributed to the putative adhesive properties of the molecule, but in retrospect could easily have been due to introduction of an inhibitory receptor. In this regard, it is notable that NIH/3T3 cells expressing PECAM-1^{Y686F}, a non-SHP-2-binding variant of PECAM-1 (14), migrate almost normally (28, 29), suggesting that inhibition of cell migration by PECAM-1 is a function of its cytoplasmic ITIM rather than its extracellular domain. Second, PECAM-1 is lost from the surface of the majority of CD4⁺ (30–33) and half of CD8⁺ (30, 31) T lymphocytes as they make the transition from naive to memory cells. Furthermore, PECAM-1-negative CD4⁺ T cells respond better to recall antigens, secrete more interleukin-4 (IL-4), and provide

better help for B-cell immunoglobulin production than do PECAM-1-positive CD4⁺ T cells (33). This is consistent with PECAM-1 playing a suppressive role in T-cell effector function. Third, although several laboratories, including my own, have reported that anti-PECAM-1 monoclonal antibodies, when bound to the cell surface, seemingly cause activation of β_1 (30, 34), β_2 (35–37), and β_3 (21) integrins, it is possible that this phenomenon is simply due to antibody-mediated sequestration of an inhibitory receptor (PECAM-1/SHP-2) away from activatory receptors that it normally regulates, resulting in a cell with increased adhesive properties. A similar explanation has been proposed for the action of anti-CD22 monoclonal antibodies, which activate B-cells, even though CD22 is now known to be an ITIM-containing negative regulator of B cell function (reviewed in ref. 38). Finally, we have recently found that PECAM-1 inhibits T-cell receptor-mediated release of calcium from intracellular stores (39) and may also function to shut down the mitogen-activated protein kinase pathway in cells after serum stimulation (Wang, R., and Newman, P.J., unpublished observations). Given these observations and the structural similarities of the PECAM-1 protein and gene to other ITIM-bearing inhibitory receptors, we propose that PECAM-1 be reassigned to the Ig-ITIM family.

What might be the implications for the presence of a newly recognized inhibitory receptor that is expressed on the surface of circulating platelets and leukocytes, and expressed abundantly at the intercellular junctions of endothelial cells after cell-cell contact? A number of testable predictions come to mind.

First, like other members of the Ig-ITIM family, PECAM-1 may function to feedback inhibit or set thresholds for cellular activation resulting from signaling cascades that emanate from ITAM-containing receptors. For example, Fc receptors (40) and a newly recognized platelet collagen receptor (41) signal via ITAMs. Might their activating and/or adhesive functions be modulated by PECAM-1 in cells that express them? The T-cell receptor complex also contains multiple ITAM-bearing subunits (the ζ chain and the CD3

ϵ , δ , and γ subunits), the signaling patterns of which are thought to be regulated, in part, by the CTLA-4 and KIRs, each of which belong to the Ig-ITIM family (Table 1). Why should T cells express still another inhibitory receptor? The answer may lie within the extracellular domain: the homophilic nature of the PECAM-1 extracellular domain (42, 43) may serve to target phosphatase activity to specific sites within the cell that are not served by these other inhibitory receptors. It is interesting to note that CTLA-4-deficient mice have constitutively activated T cells and a lymphoproliferative disorder (44, 45). It might be instructive to see, via the generation of double

Table 2
Paired cytoplasmic ITIMs that bind SHP-1, SHP-2, or both

Protein	ITIM	- spacer -	ITIM
CD22	IsYttL	rfpemniprtgdaessemqrprtcddt	VtYsaL
ILT-3 (ILT-2 has identical ITIMs)	VtYaqL	hsftlrqkatePsqegaspap	SvYatL
KIR p50/58 family consensus	VtYaqL	nhcvftqrkitrpsqrktpptdi	IvYteL
muPIR-B (p91) ITIM pair No.2	VtYaqL	csrtlrqgaasplsqaageapeep	SvYatL
muPIR-B (p91) ITIM pair No.1	SlYasV	edmqtdedgvelnswtppedpqq	EtYaqV
BGP-1 (CD66a)	VtYstL	nfeaqqptqptsaspsltate	IiYseV
SIRP-a1 (BIT, SHPS-1) ITIM No. 2	LtYadL	dmvhlnrtpkqapkppepsf	SeYasV
SIRP-a1 (BIT, SHPS-1) ITIM No. 1	ItYadL	nlpkgkkpapqaaepnnh	TeYasI
PECAM-1 (CD31)	VqYteV	qvssaeshkdldgkddte	TvYseV

The ITIM consensus is comprised of the sequence L/I/V/S-x-Y-x-x-L/V and, following a 15–30-amino acid amino acid spacer, is normally repeated to permit high-affinity binding and activation of tandem SH2-containing protein-tyrosine phosphatases. Although both ITIMs and ITAMs contain the core sequence YxxL, they differ significantly in the spacing between phosphotyrosine residues, a feature that no doubt accounts for their differential recruitment of protein-tyrosine kinases (ITAMs) versus protein-tyrosine phosphatases (ITIMs). Except for murine PIR-B, all sequences shown are human.

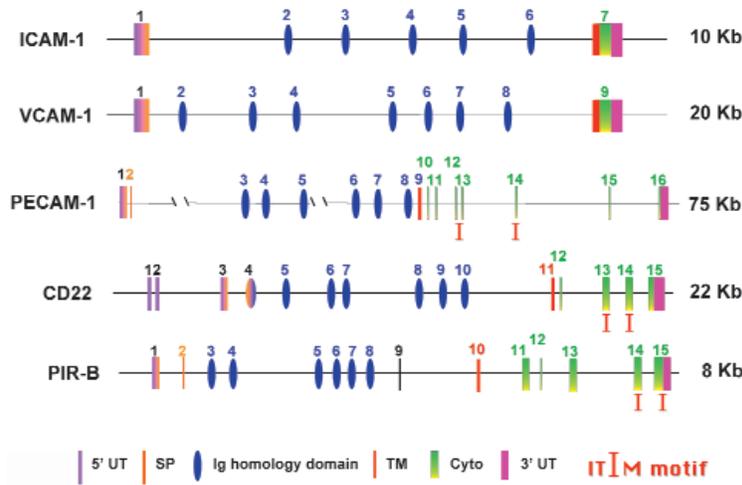


Figure 2

Organization of the genes encoding selected Ig-CAMs and Ig-ITIM family members. Note that Ig-ITIM family genes often contain a mini-exon encoding all or part of the signal peptide, and their cytoplasmic domains are encoded by multiple exons. Both of these features differ significantly from that of Ig-CAMs, in which the transmembrane and cytoplasmic domains, as well as the 3'-untranslated region, are often encoded by a single, fused exon. *ICAM-1*, intercellular cell adhesion molecule-1; *Ig-CAMs*, Ig-like cell adhesion molecules; *VCAM-1*, vascular cell adhesion molecule-1.

knockouts, whether the additional loss of PECAM-1 might lead to an even more severe phenotype.

Second, PECAM-1 is diffusely distributed on the surface of endothelial cells in culture but concentrates rapidly to cell-cell junctions once the cells have contacted each other (46). One could easily envision a scenario in which cell-cell contact leads not only to redistribution of PECAM-1 but also coincides with a transient tyrosine phosphorylation event that leads to localization of SHP-2 to cell borders. Might targeting phosphatase activity to the cell borders somehow contribute to the poorly understood phenomenon of contact inhibition? Further studies employing a constitutively active PECAM-1/SHP-2 chimeric protein may shed light on this question.

Third, PECAM-1 becomes rapidly tyrosine phosphorylated and binds SHP-2 after integrin-mediated adhesion of platelets and endothelial cells to a variety of extracellular matrix proteins (47), suggesting that PECAM-1 may function to limit the intensity and/or duration of integrin-mediated adhesion and signaling during such processes as thrombus formation and angiogenesis.

Finally, many members of the Ig-ITIM family have recently been shown to have closely related cousins, the extracellular domains of which share up to 96% sequence similarity, but whose cytoplasmic domains are devoid of ITIMs (48, 49). Thus, *FcγRIIb* (CD32), with its cytoplasmic ITIM, binds IgG and shuts down signaling, whereas its nearly identical but ITAM-containing cousin, *FcγRIIa* (paradoxically, also known as CD32), has the opposite effect. Similarly, the action of KIRs may be countered by killer activatory receptors (KARs), PIR-B by PIR-A, *ILT-2* and *ILT-3* by *ILT-1*, and *SIRP-α* by *SIRP-β* (48). Whether PECAM-1 has an antagonistic activatory counterpart (PECAM-2?) and how, in general, inhibitory receptors interact with activating receptors to regulate signaling pathways in blood and vascu-

lar cells during such events as thrombosis, inflammation, angiogenesis, and the immune response remain fruitful areas for future investigation.

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