Expression Pattern of Alternatively Spliced PECAM-1 Isoforms in Hematopoietic Cells and Platelets

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Abstract PECAM-1 (CD31) is a cell adhesion molecule that is highly expressed in the endothelium. Hematopoietic cells including platelets, monocytes, neutrophils, and some T cells also express moderate levels of PECAM-1. PECAM-1 undergoes alternative splicing generating a number of isoforms in the endothelium. However, the expression of PECAM-1 isoforms in hematopoietic cells and platelets has not been determined. Here, we examined the expression pattern of PECAM-1 isoforms in human and rodent hematopoietic cells and platelets by RT-PCR and DNA sequencing analysis. Our results showed that multiple PECAM-1 isoforms are expressed in a cell-type and species-specific pattern. We identified seven human PECAM-1 isoforms, six murine PECAM-1 isoforms, and four rat PECAM-1 isoforms. The full-length PECAM-1 was the predominant isoform detected in human cells. The PECAM-1 isoforms that lack exon 14 and 15 (Δ14&15) or Δ12,14&15 were the predominant isoform in rodent cells. In addition, we identified a novel PECAM-1 isoform, Δ13&14, in human hematopoietic cells. Thus, hematopoietic cells express multiple isoforms of PECAM-1 in a pattern similar to that observed in the endothelium of the same species. The regulated expression of these isoforms may be important during hematopoiesis and transendothelial migration. J. Cell. Biochem. 87: 424–438, 2002. © 2002 Wiley-Liss, Inc.

Key words: CD31; alternative splicing; angiogenesis; adherens junctions; cell–cell interactions

PECAM-1 (CD31) is a member of the immunoglobulin gene superfamily that is expressed on the surface of endothelial cells (EC), hematopoietic cells, and their precursor cells. In EC, PECAM-1 mediates homophilic interactions and generally localizes to the sites of cell–cell contacts [Sheibani and Frazier, 1999]. The cytoplasmic domain of PECAM-1 undergoes alternative splicing generating a number of isoforms with different adhesive properties [Yan et al., 1995; Sheibani et al., 1999]. We have shown that multiple isoforms of PECAM-1 are present in the human and murine endothelium [Sheibani et al., 1999; Wang et al., 2002] that

can be expressed in a developmentally regulated fashion [Sheibani et al., 1999]. These data suggest that PECAM-1 isoforms have different functional roles during vasculature development.

During early hematopoietic development PECAM-1 is highly expressed on CD34 enriched human hematopoietic progenitor cells and its expression is greatly reduced in more mature stages of all lineages that lack CD34 [Watt et al., 1993]. PECAM-1 expression is also regulated during differentiation and/or activation of hematopoietic cells in culture. Activation of granulocytes by FMLP (fMet-Leu-Phe) [Stockinger et al., 1990], and T cells by PHA (phytohemagglutinin) [Zehnder et al., 1992] leads to down-regulation of PECAM-1 expression. However, PMA (phorbol myristate acetate) induced differentiation of leukemia HEL and U937 cells results in up-regulation of PECAM-1 expression [Goldberger et al., 1994a]. TGFβ1-treated U937 cells also exhibit up-regulation of PECAM-1 expression [Lastres et al., 1994]. However, PECAM-1 expression is not affected in monocytes that are stimulated with FMLP [Stockinger et al., 1990], or PMA [Goldberger et al.,

1994a]. The physiological consequences of these changes and the mechanisms involved remain largely unknown.

In contrast to EC, where PECAM-1 mediates homophilic interactions, PECAM-1 expressed in hematopoietic cells generally does not participate in homophilic cell–cell interactions. Although, PECAM-1 mediated homophilic interactions between EC and leukocytes play an important role in transendothelial migration of leukocytes, both in vitro [Albelda et al., 1991; Muller et al., 1993; Muller, 1995; Berman et al., 1996] and in vivo [Vaporciyan et al., 1993, Muller, 1995]. This appears to involve both homophilic [Muller et al., 1989; Albelda et al., 1990; Newman et al., 1990; Sun et al., 1996; Newton et al., 1997] and heterophilic [Albelda et al., 1991; Muller et al., 1992; DeLisser et al., 1993; Piali et al., 1995; Buckley et al., 1996] interactions of PECAM-1 molecules on EC and hematopoietic cells. Indeed, the major phenotype of PECAM-1 null mice is delayed diapedesis of leukocytes [Duncan et al., 1999] and abnormal vascular permeability during an inflammatory challenge [Graesser et al., 2002]. Thus, PECAM-1 mediated cell–cell interactions play an important role during inflammation.

Antibodies to PECAM-1 inhibit angiogenesis in vitro [Sheibani et al., 1997; Cao et al., 2002] and in vivo [DeLisser et al., 1997] and block neutrophil and monocyte chemotaxis [Muller et al., 1993; Muller, 1995; Wakelin et al., 1996; Nakada et al., 2000] by interfering with cell–cell interactions. In addition, antibody ligation of PECAM-1 on the surface of hematopoietic cells modulates integrin activity [Tanaka et al., 1992; Piali et al., 1993; Leavesley et al., 1994; Berman and Muller, 1995; Berman et al., 1996; Chiba et al., 1999; Reedguist et al., 2000] through an inside-out signaling [Jackson et al., 1997a; Varon et al., 1998; Reedguist et al., 2000; Zhao and Newman, 2001]. The identity of these signaling pathways remains largely unknown. Thus, PECAM-1 plays multiple roles in modulating cell adhesive properties of endothelial and hematopoietic cells.

Although the hematopoietic cells are shown to express PECAM-1 on their cell surface, the identity of the PECAM-1 isoforms and the role they play in hematopoiesis and inflammation remain unknown. To gain a better understanding of the role PECAM-1 plays in these processes we determined the distribution of PECAM-1 isoforms in various hematopoietic cells and platelets. We show these cells express multiple isoforms of PECAM-1 in a species and lineage specific manner. To our knowledge this is the first report of the expression of PECAM-1 isoform in human and rodent hematopoietic cells and platelets.

MATERIALS AND METHODS

Cell Lines

The hematopoietic cell lines, human erythroleukemia (HEL) cells, human macrophage U937 cells, human T lymphocyte (lymphoblast) Jurkat cells, murine leukemia WEHI-3 cells (from BALB/c), murine cytotoxic T lymphocyte CTLL-2 cells (from C57BL/6), and rat leukemia RBL-2H3 cells (from Wistar) were obtained from the American Type Culture Collection (Rockville, MD), and maintained as recommended by the supplier. The human umbilical vein endothelial cell (HUVEC) and mouse brain endothelial cells (bEND) were maintained as previously described [Sheibani et al., 1999]. The primary human T lymphocytes were separated from peripheral blood as described by Sebzda et al. [2002].

Human and murine platelets were isolated from blood samples as described previously [Chung et al., 1997]. Briefly, human blood was obtained from healthy human donors in 0.15 volumes of acid-citrate/dextrose solution supplemented with 1 mM prostaglandin E1 and 1 U/ml apyrase. Mouse blood was obtained from 8–10 adult mice of the C129 or FVBN strains. The platelet-rich plasma was separated from whole blood at 500 g in a Beckman GP tabletop centrifuge for 30 min, followed by centrifugation at 1000 g for 30 min to pellet the platelets. Platelets were washed and lyzed for total RNA isolation using RNAWIZ (Ambion) as recommended by the supplier.

Determination of the Alternatively Spliced PECAM-1 Isoforms

Poly A^+ RNA was isolated from different human and murine cell lines as described previously [Sheibani et al., 1991]. The mRNAs were utilized as template for RT-PCR (Superscript^{TM} One-Step RT-PCR; Gibco-BRL, Gaithersberg, MD) to amplify the cytoplasmic domain of all possible PECAM-1 isoforms. The sense primers were designed as 5′-atggatcc[2021]AGG AAA GCC

AAG GCC AGG[2038]-3′ for human PECAM-1, and 5′-atggatcc[1941]AGG AAA GCC AAG GCC AAA[1958]-3′ for murine PECAM-1, which spans the border of exon 9 and exon 10 within the intracellular domain. The anti-sense primers were designed as 5′-cggaattc[2371]CCT TGC TGT CTA AGT CCT[2354]-3′ for human PECAM-1, and 5′-cggaattc[2291]TTG ACT GTC TTA AGT TCC[2274]-3′ for murine PECAM-1, which spans the border of exon 16 and 3′-untranslated region. For primary human T lymphocytes, direct capture of poly A$^+$ RNA and RT-PCR was performed as recommended by the supplier (ExpressDirect™ mRNA capture and RT system for RT-PCR, Pierce, Rockford, IL). The primers carry a BamHI and an EcoRI recognition sequence (lowercase letters) to facilitate subsequent cloning. PCR products were examined on 2.4% agarose gels to assess their integrity and expected size. For cloning, PCR products were purified, digested with BamHI and EcoRI, ligated into the pGEX-2T vector (Pharmacia) cut with the same enzymes, and transformed into *E. coli* DH5α. Bacterial colonies were screened by BamHI and EcoRI digestion of minipreps and those with inserts were sequenced using the Big Dye (University of Wisconsin Biotechnology Center). Identification of PECAM-1 isoforms was performed as described previously [Sheibani et al., 1999; Wang et al., 2002].

Northern Blot Analysis

Poly A$^+$ RNA (5 μg) isolated from HUVEC, bEND and RBL-2H3 cells [Sheibani et al., 1991] was size fractionated in a 1.2% agarose formaldehyde gel, transferred to zeta-probe membrane (Bio-Rad), pre-hybridized, and hybridized to random primer ^{32}p-labeled full-length cDNA probes for mouse (obtained from Dr. Albelda, University of Pennsylvania Medical Center) and human PECAM-1 (obtained from Dr. Newman, Blood Research Institute of Southeastern Wisconsin). The blots were also probed with a cDNA for GAPDH to control for loading. Please note that the exposure time varied for each probe.

Cloning of Partial Rat PECAM-1 cDNA and Identification of the Alternatively Spliced Rat PECAM-1 Isoforms

Poly A$^+$ RNA was isolated from rat hematopoietic RBL-2H3 cells as described above and utilized for RT-PCR to clone partial cDNA of rat PECAM-1 using different sets of primers derived from mouse PECAM-1 cDNA sequence. The first set of the sense primer was designed as 5′-atggatcc[1749]GCT AGC AAG AAG CAG GAA[1766]-3′, which spans the border of exon 8 within the extracellular domain, and anti-sense primer 5′-cggaattc[2291]TTG ACT GTC TTA AGT TCC[2274]-3′, which spans the border of exon 16 and 3′-untranslated region. The second set of sense primer, 5′-[1398]ATG AAA GCA AAG AGT GAC[1415]-3′, which spans the border of exon 7 within the extracellular domain, flanking the BstE II restriction site (the internal BstEII site of murine PECAM-1 cDNA) within the primer and anti-sense primer 5′-cgaatgc[2351]AT CCA GGA ATC GGC TGC TCT TC[2329]-3′, which spans the border of 3′-untranslated region, carrying a 5′Nsi I recognition site for cloning purposes. The expected sizes of the RT-PCR products are ∼600 and 900 bp, respectively (see Fig. 5). The 600 bp fragment with BamHI and EcoRI sites was cloned into pGEX-2T cut with the same enzymes. The ∼900 bp fragment with the NsiI and BstEII recognition sites was cloned into the plasmid pcDNAIneo (Invitrogen) carrying mouse Δ15 PECAM-1 cut with the same NsiI and BstEII to replace the mouse Δ15 cytoplasmic domain with rat cytoplasmic domain. DNA sequencing of these constructs showed that there is only a single nucleotide difference between the rat and murine primer sequences in the cytoplasmic region (Fig. 6). To identify rat PECAM-1 isoforms in the RBL-2H3 cells, the same mRNA sample was utilized as template for RT-PCR to amplify all possible rat PECAM-1 isoforms, using the murine primers that were used to amplify the murine PECAM-1 isoforms. The expected size of the RT-PCR product is about 350 bp. The 350 bp fragment with BamHI and EcoRI sites was cloned into plasmid pGEX-2T. The cloning of the rat PECAM-1 cDNA fragments and sequencing were performed as described above.

The putative full-length rat PECAM-1 cDNA was derived from the various rat PECAM-1 isoforms. The rat PECAM-1 isoforms that lack exon 12, 15, 14&15, and 12, 14&15 were identified. The lack of the exon 15 in Δ15 and Δ14&15 isoforms results in a change in the reading frame (see Figs. 7 and 8). Identification of rat PECAM-1 isoforms was performed by comparison to mouse PECAM-1 cDNA [Xie and Muller, 1993; Sheibani et al., 1999].

Expression of PECAM-1 Isoforms

U937 cells express moderate levels of PECAM-1 on their surface [Goldberger et al., 1994a; Wang et al., 2002]. The PECAM-1 level in these cells is modulated by incubation with PMA [Goldberger et al., 1994a; Wang et al., 2002], promoting the differentiation of these cells towards a macrophage lineage. These cells express multiple isoforms of PECAM-1. In addition, the expression pattern of the PECAM-1 isoforms changes upon incubation of these cells with PMA (Wang and Sheibani, unpublished data). To demonstrate that the product of the different PECAM-1 isoform is translated we examined the cell lysates prepared from PMA or DMSO (control) treated U937 cells by Western blotting. U937 cells were incubated with 20 nM PMA (Calbiochem, Dan Diego, CA) or DMSO (solvent control) for different times. Following PMA treatment, cells were pelleted, washed with cold TBS (20 mM Tris-HCl, 150 mM NaCl, pH 7.4), lyzed in 0.5 ml of lysis buffer (20 mM Tris-HCl, pH 7.4, 2 mM EDTA, and protease inhibitors cocktail (Roch Biochemicals) with a brief sonication. Protein concentrations were determined using the BCA protein assay kit (Pierce). Equal amount of protein lysates (25 μg) were analyzed by SDS–PAGE (4–20% Tris-Glycine gel, Invitrogen), transferred to Nitrocellulose, and blotted with either an antibody made to the extracellular domain of human PECAM-1 (recognizes all PECAM-1 isoforms; SEW 16, a gift from Dr. Peter Newman) or an antibody made to the exon 14 of murine PECAM-1 (recognizes isoforms that contain exon 14; Sheibani et al., 1999). Following incubation with appropriate secondary antibodies, blots were washed, and developed using ECL (Amersham).

Identification of the Alternatively Spliced PECAM-1 mRNA Variants Loaded on Polysomes for Translation

Polysomes were isolated from HEL cells using modification of published protocols [Lindeman et al., 2001]. In brief, the cells (5×10^7) were lyzed in the RSB solution (10 mM Tris-acetate, pH 7.4, containing 0.1 mM potassium acetate, 2.25 mM magnesium acetate, and 2 mM dithiotriethnol). The lysate was centrifuged at 14,000g for 10 min at 4°C to remove the cell debris. The resulting supernatant was placed on a 4 ml 30% sucrose pad and centrifuged at 4°C for 2.5 h (175,000g). The polysome pellets were washed twice with the RSB solution, and used for direct capture of poly A$^+$ RNA and RT-PCR analysis as recommended by the supplier (ExpressDirect^{TM} mRNA capture and RT system for RT-PCR, Pierce). The RT-PCR product amplified from the polysome poly A$^+$ RNA was cloned into plasmid pGEX-2T and sequenced. Determination of the alternatively spliced PECAM-1 mRNA variants in polysome mRNA was performed as described above.

RESULTS

Distribution of PECAM-1 Isoforms in Hematopoietic Cells

PECAM-1 plays an important role in angiogenesis and inflammation. Mice that lack PECAM-1 develop normally but exhibit defects in diapedesis of leukocytes [Duncan et al., 1999] and aberrant permeability in an inflammatory challenge [Graesser et al., 2002]. Although PECAM-1 is expressed on the surface of hematopoietic cells, it normally does not participate in homophilic and/or heterophilic cell–cell interactions as observed in EC. This is perhaps due to the presence of different PECAM-1 isoforms and/or different regulatory mechanisms, which operate in these cells. Here, we have determined the expression pattern of PECAM-1 isoforms in various human and murine hematopoietic cells including platelets and primary T lymphocytes by RT-PCR cloning and DNA sequencing analysis. The primers were designed to encompass the entire PECAM-1 cytoplasmic domain and amplify all PECAM-1 isoforms.

Figure 1 illustrates the RT-PCR products generated from human and murine hematopoietic cells and platelets. Most RNA samples from human cells exhibited a predominant band corresponding to the expected full-length PECAM-1, with the exception of HEL cells, which exhibited multiple bands. In contract, the murine cells exhibited multiple bands. These data are consistent with the RT-PCR patterns previously detected in human and murine endothelium, respectively [Sheibani et al., 1999; Wang et al., 2002]. Alternative splicing of the murine PECAM-1 mRNA is more prevalent than human PECAM-1 mRNA. We also observed that different mouse strains exhibited different PECAM-1 expression patterns. C129 mouse platelets showed a strong smaller band and a weak upper band, which is different from the
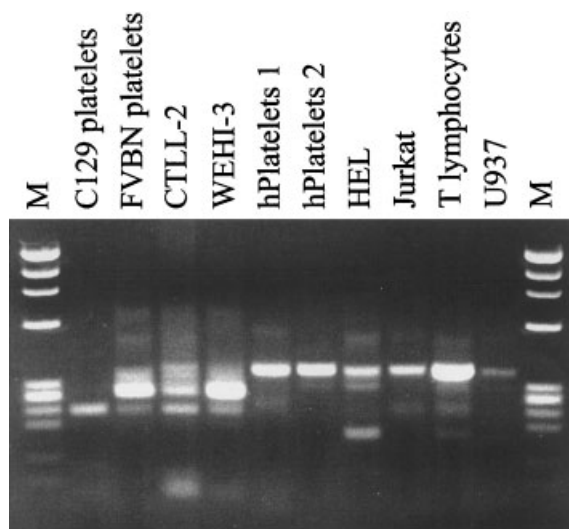
Fig. 1. The RT/PCR analysis of RNA isolated from human and murine hematopoietic cells. The RT-PCR products amplified from RNA isolated from the human and murine lymphocytic cells and platelets were separated on 2.4% agarose gels. M designates the molecular marker and the bands correspond to 1350, 1078, 872, 603, 310, 280, 234, 194, and 72 bp, respectively. These experiments were repeated with two-separate preparations of RNA with identical results.

expressed in the hematopoietic cells and platelets. Seven PECAM-1 isoforms were detected in human cells (Table I). These included the full-length PECAM-1 and isoforms that either lack exon 12, 13, 14, 15, 13&14, or 14&15. Six PECAM-1 isoforms were detected in murine cells (Table II). These included the full-length PECAM-1 and isoforms that either lack exon 14, 15, 12&14, 14&15, or 12,14&15. The PECAM-1 isoforms that lack exon 12, 13, or 13&14 were not detected in the murine cells, while the PECAM-1 isoforms that lack exon 12&14, or 12,14&15 were not detected in the human cells. The full-length PECAM-1 isoform is the predominant isoform detected in human cells. However, the isoforms Δ14&15 or Δ12,14&15 were the predominant isoforms in the FVBN mouse platelets (Δ14&15; 64%), WEHI-3 cells (Δ14&15; 87%), C129 mouse platelets (Δ12, 14&15; 94%), and CTLL-2 cells (Δ12,14&15, 43%), respectively. We did not detect the full-length PECAM-1 in the C129 platelets and WEHI-3 cells. The isoform Δ15 is the second predominant isoform in mouse endothelial cells [Sheibani et al., 1999]. Mouse hematopoietic cells express this isoform at lower frequencies (Table II). The Δ15 PECAM-1 isoform was detected in all the human cells, whereas the Δ14&15 and Δ12,14&15 isoforms were detected in all the murine cells. Human HEL, Jurkat, and U937 cells expressed a greater number of PECAM-1 isoforms. Human platelets and primary T lymphocytes expressed fewer numbers of PECAM-1 isoforms. This is very similar to the pattern of PECAM-1 isoforms in human endothelium [Wang et al., 2002]. Therefore, it appears that PECAM-1 isoforms are expressed in species and cell-type specific manner. A novel PECAM-1 isoform [Δ13&14], which has not been previously detected in human endothelium, was detected in HEL and U937 cells. Its cDNA and amino acid sequence are shown in Figures 2 and 3, respectively.

pattern detected in the FVBN mouse platelets. FVBN mouse platelets, WEHI-3, and CTLL-2 cell lines showed a more similar pattern of multiple bands with variable intensity. Therefore, these cells may express PECAM-1 isoforms at variable frequencies. However, it is not possible to differentiate all the potential PECAM-1 isoforms at this resolution.

Identification of PECAM-1 Isoforms in Human and Murine Hematopoietic Cells and Platelets

The identity of the cDNAs generated by RT-PCR was determined by direct cloning and sequencing as described in Materials and Methods. Tables I and II show that multiple isoforms of human and murine PECAM-1 are

TABLE I. Distribution of PECAM-1 Isoforms in Human Hematopoietic Cells

PECAM-1 isoforms	Full[a]	Δ12	Δ13	Δ14	Δ15	Δ12&14	Δ13&14	Δ14&15	Δ12,14&15
Platelet (28)	96	ND	ND	ND	4	ND	ND	ND	ND
HEL (27)	33	ND	7	30	19	ND	7	4	ND
Jurket (25)	76	4	ND	12	8	ND	ND	ND	ND
T cell (30)	93	ND	ND	ND	7	ND	ND	ND	ND
U937 (27)	67	ND	ND	22	7	ND	4	ND	ND

Isoforms of PECAM-1 were identified by cloning and sequencing RT-PCR products from mRNA isolated from human platelets, primary T lymphocyte and cultured human lymphocytic cell lines as described in the Materials and Methods. The numbers in parentheses indicate the total number of PECAM-1 clones examined. ND, not detected.
[a]The number indicates the frequency in percent at which each isoform was detected.

TABLE II. Distribution of PECAM-1 Isoforms in Murine Hematopoietic Cells

PECAM-1 isoforms	Full[a]	Δ12	Δ13	Δ14	Δ15	Δ12&14	Δ14&15	Δ12,14&15
Platelet (C129) (31)	ND	ND	ND	ND	ND	ND	6	94
Platelet (FVBN) (25)	16	ND	ND	4	8	ND	64	8
CTLL-2 (C57BL/6) (21)	5	ND	ND	29	10	5	10	43
WEHI-3 (BALB/c) (23)	ND	ND	ND	9	ND	ND	87	4

Isoforms of PECAM-1 were identified by cloning and sequencing RT-PCR products from RNA isolated from murine platelets and lymphocytic cell lines as described in the Materials and Methods. The numbers in parentheses indicate the total number of PECAM-1 clones examined. ND, not detected.
[a]The number indicates the frequency in percent at which each isoform was detected.

Partial cDNA Sequence of Rat PECAM-1 and Its Alternatively Spliced Isoforms

Rat PECAM-1 cDNA has remained uncharacterized. A partial cDNA sequence (1026 bp) of rat PECAM-1 is available in the NCBI GeneBank [Mandriota and Pepper, 1996] does not include the sequence of rat PECAM-1 cytoplasmic domain. We had previously noticed that, despite great degree of homology between murine and human PECAM-1, their full-length cDNA probes fail to equally cross-hybridize in Northern blots. Figure 4 shows a Northern blot of RNA isolated from HUVEC (human EC), bEND (murine EC) and RBL-2H3 (rat hematopoietic cells). The results show that murine PECAM-1 cDNA probe is able to cross hybridize well with rat PECAM-1 cDNA mRNA, but not with human PECAM-1 mRNA, while the human PECAM-1 cDNA probe is not able to cross hybridize with rat PECAM-1 mRNA and minimally with murine PECAM-1 mRNA. Murine PECAM-1 cDNA probe gave stronger signal than the human cDNA probe confirming greater degree of homology between rat and murine PECAM-1 cDNAs.

We next utilized the murine PECAM-1 primers to clone the cytoplasmic domain of rat PECAM-1 cDNA using RT-PCR. Two sets of primers were used to generate two different size fragments of rat PECAM-1 cDNA (Fig. 5). A 600 bp fragment, which revealed the sequence information expanding exon 8 through 15 and part of exon 16. A 900 bp fragment which revealed the nucleotide sequence that verifies the detected sequence in the ~600 bp fragment, and especially part of exon 16 and 3'-untranslated region (the anti-sense primer region for the ~600 bp fragment). The partial cDNA sequence of rat full-length PECAM-1 cDNA (exons 8–16) is shown in Figure 6, which was derived from the sequences of alternatively spliced rat PECAM-1 isoforms (Fig. 7). The cDNA sequence expands putative exons 8, 9 (transmembrane domain), and exons 10–16 (cytoplasmic domain and termination codon) of rat PECAM-1. Based on our sequence data (Fig. 6), there is a 92% sequence homology between murine and rat PECAM-1 cDNAs, and 78% homology between human and rat PECAM-1 cDNA in the examined region. This is in agreement with the Northern analysis data

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Full        AGG AAA GCC AAG GCC AAG CAG ATG CCA GTG GAA ATG TCC AGG CCA GCA GTA CCA CTT CTG ACC TCC
Δ13&14      AGG AAA GCC AAG GCC AAG CAG ATG CCA GTG GAA ATG TCC AGG CCA GCA GTA CCA CTT CTG ACC TCC

AAC AAC GAG AAA ATG TCA GAT CCC AAT ATG GAA GCT AAC AGT CAT TAC GGT CAC AAT GAC GAT GTC AGA AAC CAT
AAC AAC GAG AAA ATG TCA GAT CCC AAT ATG GAA GCT AAC AGT CAT TAC GGT CAC AAT GAC GAT GTC GGA AAC CAT

GCA ATG AAA CCA ATA AAT GAT AAT AAA GAG CCT CTG AAC TCA GAC GTG CAG TAC ACG GAA GTT CAA GTG TCC TCA
GCA ATG AAA CCA ATA AAT GAT AAT AAA G-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

GCT GAG TCT CAC AAA GAT CTA GGA AAG AAG GAC ACA GAG ACA GTG TAC AGT GAA GTC CGG AAA GCT GTC CCT GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -AT

GCC GTG GAA AGC AGA TAC TCT AGA ACG GAA GGC TCC CTT GAT GGA ACT TAG ACA GCA AGG
GCC GTG GAA AGC AGA TAC TCT AGA ACG GAA GGC TCC CTT GAT GGA ACT TAG ACA GCA AGG
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Fig. 2. The cDNA sequence of the human Δ13&14 PECAM-1 cytoplasmic domain. The nucleotide sequence of the human PECAM-1 isoform lacking exons 13 and 14 is compared to the full-length human PECAM-1 cytoplasmic domain. The deleted sequences are indicated by hyphens (-). The predicated exon sequences of human PECAM-1 were adapted from Newman et al. [1990].

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Full            R K A K A K Q M P V E M S R P A V P L L N S N
Δ13&14          R K A K A K Q M P V E M S R P A V P L L N S N

N E K M S D P N M E A N S H Y G H N D D V R N H A M K P I N D
N E K M S D P N M E A N S H Y G H N D D V R N H A M K P I N D

N K E P L N S D V Q Y T E V Q V S S A E S H K D L G K K D T E
N K - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

T V Y S E V R K A V P D A V E S R Y S R T E G S L D G T *
- - - - - - - - - - - D A V E S R Y S R T E G S L D G T *
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Fig. 3. The amino acid sequence of the human Δ13&14 PECAM-1 cytoplasmic domain. The amino acid sequence of the human PECAM-1 isoform lacking exon 13 and 14 is compared with full-length human PECAM-1 cytoplasmic domain. The deleted sequences are indicated by hyphens (-). The putative exon sequences of human PECAM-1 were indicated according to the cDNA sequence data (above).

(Fig. 4), which showed stronger hybridization between murine and rat compared with human and rat PECAM-1 RNA/cDNA. There is a single codon deletion in the exon 8 of the rat full-length PECAM-1, compared to the cDNA of murine PECAM-1 (Fig. 6) by a sequence alignment analysis. The putative partial amino acid sequence of rat PECAM-1 (Fig. 6) revealed that there is a 76% similarity between murine and rat PECAM-1, and 60% between human and rat PECAM-1 in the examined region. Thus, there are significant differences in the primary structure of mouse and rat compared to human PECAM-1 mRNA. The reported partial cDNA sequence of rat PECAM-1 [1026 bp, Mandriota
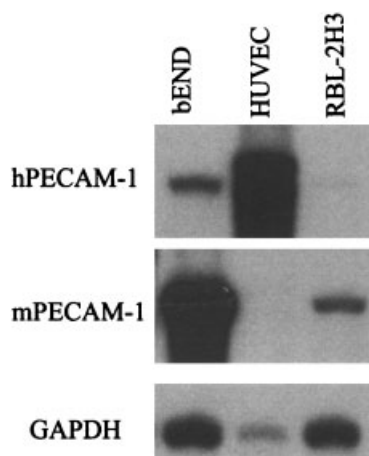

Fig. 5. The RT-PCR analysis of RNA isolated from rat RBL-2H3 cells. The RT-PCR products were amplified from RNA isolated from the RBL-2H3 cells as described in Materials and Methods. The PCR products were separated on 2.4% agarose gels, stained with ethidium bromide, and photographed. M designates the molecular weight marker and the bands correspond to 1350, 1078, 872, 603, 310, 280, 234, 194, and 72 bp, respectively. These experiments were repeated with two separate preparations of RNA with identical results.

Fig. 4. Northern blot analysis of PECAM-1. Poly A$^+$ RNA (5 μg) isolated from HUVEC (human EC), bEND (mouse EC), and RBL-2H3 (rat leukemia) cells was size fractionated in a 1.2% agarose-formaldehyde gel, transferred to zeta-prob membrane (Bio-Rad), prehybrized, and hybridized to random primer ^{32}P-labeled full-length cDNA probes for human and mouse PECAM-1. Blots were also probed with a cDNA for GAPDH to control for loading. Please note the exposure time varied for each probe.
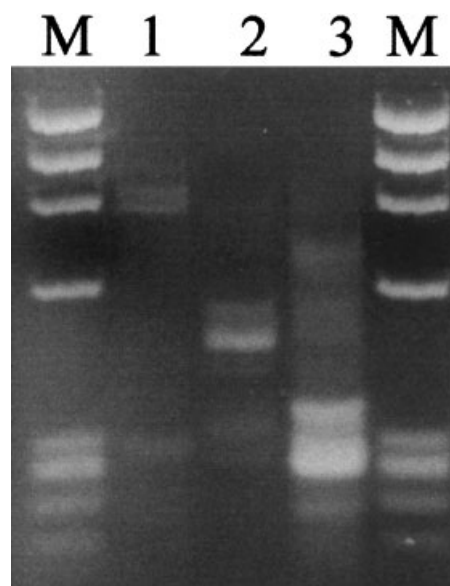
and Pepper, 1996] spans part of exon 5, exons 6 through 9, and part of exon 10 in rat PECAM-1. The putative exon identification was performed by comparison to murine PECAM-1 [Xie and Muller, 1993]. The overlapping sequence (222 bp), between the sequence presented here and that of Mandriota and Pepper [1996] indicated a 99% similarity. The minor differences could be attributed to mutation or analysis error, as has been observed in PECAM-1 cDNA sequence from HUVEC [Newman et al., 1990; Zehnder et al., 1992].

We determined the identity of rat PECAM-1 isoforms expressed in rat RBL-2H3 cells. The RT-PCR results from RBL-2H3 cells showed the pattern of multiple bands similar to that observed in mouse WEHI-3 cells and the FVBN mouse platelets (Figs. 1 and 5). Cloning and sequencing data indicated that the RBL-2H3 cells express four different PECAM-1 isoforms including Δ12, Δ15, Δ14&15, and Δ12,14&15 at variable frequencies (Table III). The Δ14&15 isoform, like in murine endothelium and hematopoietic cells, is the predominant isoform detected. This is the first evidence showing the alternatively splicing of rat PECAM-1.

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hPECAM-1    GCT AGC AAG GAA CAG GAG GGA GAG TAT TAC TGC ACA GCC TTC AAC AGA
mPECAM-1    GCT AGC AAG AAG CAG GAA GGA CAG TAC TAC TGT ACA GCC TCC AAC AGA
rPECAM-1    GCT AGC AAG AAG CAG GAA GGC CAG TAC TAT TGT ACA GCG TTC AAC AGA
rPECAM-1     A   S   K   K   Q   E   G   Q   Y   Y   C   T   A   F   N   R

            GCC AAC CAC GCC TCC AGT GTC CCC AGA AGC AAA ATA CTG ACA GTC AGA GTC ATT CTT
            GCC AGC AGT ATG AGG ACC AGT CCC CGA AGC AGC ACT CTT GCA GTC AGA GTC TTC CTT
            GCC AGC ATT GTG     ACC AGT CTC CGA AGC GGC CCT CTA ACA GTC AGA GTC TTT CTT
             A   S   I   V       T   S   L   R   S   G   P   L   T   V   R   V   F   L

            GCC CCA TGG AAG AAA GGA CTT ATT GCA GTG GTT ATC ATC GGA GTG ATC ATT GCT CTC
            GCC CCA TGG AAG AAA GGG CTC ATT GCG GTG GTT GTC ATT GGA GTG GTC ATC GCC ACC
            GCT CCG TGG AAG AAA GGG CTC ATT GCA GTC GTT GTC ATT GGA GTG GTC ATT GCC GCC
             A   P   W   K   K   G   L   I   A   V   V   V   I   G   V   V   I   A   A

            TTG ATC ATT GCG GCC AAA TGT TAT TTT CTG AGG AAA GCC AAG GCC AAG CAG ATG CCA
            TTA ATA GTT GCA GCC AAA TGC TAC TTC CTG AGG AAA GCC AAG GCC AAA CAG AAA CCC
            TTG ATA GTT GCA GCC AAA TAC TAC TTC CTG AGG AAA GCC AAG GCC AAG CAG AAG CCC
             L   I   V   A   A   K   Y   Y   F   L   R   K   A   K   A   K   Q   K   P

            GTG GAA ATG TCC AGG CCA GCA GTA CCA CTT CTG AAC TCC AAC AAC GAG AAA ATG TCA
            GTG GAG ATG TCC AGG CCA GCT GCT CCA CTT CTG AAC CCC AAC AGC GAG AAG ATT TCT
            GTG GAG ATG TCC AGG CCA GCA GTC CCA CTT CTG AAC TCC AAC AGT GAG AAG GTT TCT
             V   E   M   S   R   P   A   V   P   L   L   N   S   N   S   E   L   V   S

            GAT CCC AAT ATG GAA GCT AAC AGT CAT TAC GGT CAC AAT GAC GAT GTC AGA AAC CAT
            GAG CCT AGT GTG GAA GCC AAC AGC CAT TAC GGT TAT GAT GAT GTT TCT GGA AAT GAT
            GAG CCC AGT GTG GAA ACC AAC AGC CAT TAC GGT TAT GAT GAT GTT TCT GGA ATT GAT
             E   P   S   V   E   T   T   S   H   Y   G   Y   D   D   V   S   E   I   D

            GCA ATG AAA CCA ATA AAT GAT AAT AAA GAG CCT CTG AAC TCA GAC GTG CAG TAC ACG
            GCA GTA AAA CCC ATA AAT CAA AAT AAA GAC CCC CAG AAC ATG GAT GTA GAA TAC ACA
            GCA GTA AAA CCC ATA AAT CAA AAT AAA GAC TCC CAG AAC ATG GAT GTG GAG TAC ACA
             A   V   K   P   I   N   Q   N   K   D   S   Q   N   M   D   V   E   Y   T

            GAA GTT CAA GTG TCC TCA GCT GAG TCT CAC AAA GAT CTA GGA AAG AAG GAC ACA GAG
            GAA GTG GAA GTG TCC TCC CTT GAG CCT CAC CAA GCT CTG GGA ACG AGA GCC ACA GAG
            GAA GTG GAA GTG TCC TCC CTT GAG CCT CAC CAA GCT CTG GGA ACG AGA GCC ACA GAG
             E   V   E   V   S   S   L   E   P   H   Q   A   L   G   T   R   A   T   E

            ACA GTG TAC AGT GAA GTC CGG AAA GCT GTC CCT GAT GCC GTG GAA AGC AGA TAC TCT
            ACG GTG TAC AGT GAG ATC CGG AAG GTC GAC CCT AAT CTC ATG GAA AAC AGA TAT TCT
            ACG GTG TAC AGT GAA ATC CGG AAG GTC GAC CCT AAT TTC GTG GGA AAC AGA TAC TCT
             T   V   Y   S   E   I   R   K   V   D   P   N   F   V   G   N   R   Y   S

            AGA ACG GAA GGC TCC CTT GAT GGA ACT TAG ACA GCA AGG
            AGA ACG GAA GGC TCC CTT AAT GGA ACT TAA GAC AGT CAA
            AGA ACG GAA GGC CTC CTT GAT GGA ACT TAA GAC AGT CAA
             R   T   E   G   L   L   D   G   T   *
```

Fig. 6. Partial cDNA and amino acid sequence of full-length rat PECAM-1 isoform. The nucleotide sequence of full-length rat PECAM-1 isoform (exons 8–16) is compared to the full-length human and mouse PECAM-1 isoforms. The predicated exon sequences of human and mouse PECAM-1 were adapted from Newman et al. [1990] and Xie and Muller [1993], respectively. Please note the deletion of a single codon in rat exon 8 cDNA sequence.

The exonic mutation sites of rat PECAM-1 cDNA molecules were identified by comparison of the mutant sequences with the wild type mouse sequence [Xie and Muller, 1993; Sheibani et al., 1999]. The Δ12 isoform with a new junction in cDNA sequence was identified at G^{2135}-A^{2190} (loss of 55 bp). The Δ15 isoform was identified at G^{2309}–A^{2333} (loss of 24 bp). The Δ14&15 isoform was identified at G^{2252}–A^{2333} (loss of 81 bp). The Δ12,14&15 isoform was identified at G^{2135}–A^{2190} (exon 12) and G^{2252}–A^{2333} (exons 14 and 15) (loss of 136 bp). The

```
Full         AGGAAAGCCAAGGCCAAGCAGAAGCCCGTGGAGATGTCCAGGCCAGCAGTCCCACTTCTGAAC
Δ12          AGGAAAGCCAAGGCCAAGCAGAAGCCCGTGGAGATGTCCAGGCCAGCAGTCCCACTTCTGAAC
Δ15          AGGAAAGCCAAGGCCAAGCAGAAGCCCGTGGAGATGTCCAGGCCAGCAGTCCCACTTCTGAAC
Δ14&15       AGGAAAGCCAAGGCCAAGCAGAAGCCCGTGGAGATGTCCAGGCCAGCAGTCCCACTTCTGAAC
Δ12,14&15    AGGAAAGCCAAGGCCAAGCAGAAGCCCGTGGAGATGTCCAGGCCAGCAGTCCCACTTCTGAAC

TCCAACAGTGAGAAGGTTTCTGAGCCCAGTGTGGAAACCAACAGCCATTACGGTTATGATGATGTTTCTGGAATT
TCCAACAGTGAGAAGGTTTCTGAGCCCAGTGTGGAAACCAACAGCCATTACG----------------------
TCCAACAGTGAGAAGGTTTCTGAGCCCAGTGTGGAAACCAACAGCCATTACGGTTATGATGATGTTTCTGGAATT
TCCAACAGTGAGAAGGTTTCTGAGCCCAGTGTGGAAACCAACAGCCATTACGGTTATGATGATGTTTCTGGAATT
TCCAACAGTGAGAAGGTTTCTGAGCCCAGTGTGGAAACCAACAGCCATTACG----------------------

GATGCAGTAAAACCCATAAATCAAAATAAAGACTCCCAGAACATGGATGTGGAGTACACAGAAGTGGAAGTGTCC
---------------------------ACTCCCAGAACATGGATGTGGAGTACACAGAAGTGGAAGTGTCC
GATGCAGTAAAACCCATAAATCAAAATAAAGACTCCCAGAACATGGATGTGGAGTACACAGAAGTGGAAGTGTCC
GATGCAGTAAAACCCATAAATCAAAATAAAGACTCCCAGAACATGGATGTGGAGTACACAGAAGTGGAAGTGTCC
---------------------------ACTCCCAGAACATGGATGTGGAGTACACAGAAGTGGAAGTGTCC

TCCCTTGAGCCTCACCAAGCTCTGGGAACGAGAGCCACAGAGACGGTGTACAGTGAAATCCGGAAGGTCGACCCT
TCCCTTGAGCCTCACCAAGCTCTGGGAACGAGAGCCACAGAGACGGTGTACAGTGAAATCCGGAAGGTCGACCCT
TCCCTTGAGCCTCACCAAGCTCTGGGAACGAGAGCCACAGAGACGGTGTACAGTGAAATCCGGAAGGTCGACCCT
TCCCTTGAGCCTCACCAAG--------------------------------------------------------
TCCCTTGAGCCTCACCAAG--------------------------------------------------------

AATTTCGTGGGAAACAGATACTCTAGAACGGAAGGCCTCCTTGATGGAACTTAAGACAGTCAA
AATTTCGTGGGAAACAGATACTCTAGAACGGAAGGCCTCCTTGATGGAACTTAAGACAGTCAA
A---------------------AGAACGGAAGGCCTCCTTGATGGAACTTAAGACAGTCAA
---------------------AGAACGGAAGGCCTCCTTGATGGAACTTAAGACAGTCAA
---------------------AGAACGGAAGGCCTCCTTGATGGAACTTAAGACAGTCAA
```

Fig. 7. The cDNA sequence of the rat PECAM-1 isoform cytoplasmic domains. The nucleotide sequences of the rat PECAM-1 isoforms lacking exon 12, 15, 14 and 15, or 12, 14 and 15 are compared to the putative full-length rat PECAM-1 isoform. The deleted sequences are indicated by hyphens (-). The predicated exon sequences of rat PECAM-1 were adapted from human and murine PECAM-1, Newman et al. [1990] and Xie and Muller [1993], respectively.

expected size for the wild type form (full-length PECAM-1 cytoplasmic domain) is 351 bp derived from the cDNA sequences of rat PECAM-1 isoforms. The absence of exon 15 in Δ15, Δ14&15, and Δ12,14&15 isoforms changes the reading frame resulting in utilization of an upstream termination codon (TGA instead of TAA) shortening the cDNA by three amino acids and incorporation of six different amino acids upstream of the termination codon (see Figs. 7 and 8). The alternative splicing sites of PECAM-1 is remarkably conserved in these three species. Please note that the numbers that indicate the new junctions of alternative splicing sites in rat cDNA are adapted from murine PECAM-1 cDNA [Xie and Muller, 1993] since the complete rat PECAM-1 cDNA sequence has not been reported.

Translation of the Alternatively Spliced PECAM-1 Isoforms

To determine whether alternatively spliced isoforms of PECAM-1 are translated, we first examined the expression pattern of PECAM-1 isoforms in human U937 cells during their macrophage differentiation. U937 cells express moderate levels of PECAM-1, which is up-regulated during PMA induced differentiation. U937 cells express full length and three additional isoforms of PECAM-1 including Δ15, Δ13&14, and Δ14 (Table I). This pattern dramatically changes upon PMA treatment and includes a number of PMA-induced isoforms, Δ13 and Δ14 (the predominant isoform detected) (Wang and Sheibani, unpublished data). Figure 9 shows the Western blot analysis of cell lysates prepared from U937 cell following incubation with PMA or DMSO (control). The blot was incubated either with an antibody to human PECAM-1 (SEW 16), which recognizes the extracellular domain of PECAM-1 (detects all isoforms of PECAM-1; Fig. 9, left panel) or an antibody to murine exon 14 (recognizes isoforms with exon 14 [Sheibani et al., 1999; Wang et al., 2002; Fig. 9, right panel]. The antibody to the extracellular domain of human PECAM-1

TABLE III. Distribution of PECAM-1 Isoforms in Rat Hematopoietic Cells

PECAM-1 isoforms	Full[a]	Δ12	Δ13	Δ14	Δ15	Δ12&14	Δ13&14	Δ14&15	Δ12,14&15
RBL-2H3 (13)	ND	8	ND	ND	15	ND	ND	54	8

Isoforms of PECAM-1 were identified by cloning and sequencing RT-PCR products from mRNA isolated from rat leukemic RBL-2H3 cells as described in the Materials and Methods. The numbers in parentheses indicate the total number of PECAM-1 clones examined. ND, not detected.
[a]The number indicates the frequency in percent at which each isoform was detected.

recognized a band corresponding to the full-length PECAM-1 (130 kDa) in DMSO treated cells. However, one additional lower molecular weight band (approximately 110–120 kDa) was also detected which becomes more prominent upon PMA treatment (Fig. 9, left panel). This band was not detected when the same blot was probed with the exon 14 antibody (Fig. 9; right panel). The exon 14 antibody only detected a band corresponding to the full-length PECAM-1 in PMA or DMSO treated cells. Thus, the lower molecular weight band is the products of PECAM-1 isoform(s), which lack exon 14. This is consistent with the pattern of PECAM-1 isoforms detected in U937 cells following PMA treatment. A similar pattern was observed in

the HEL cells (data not shown). Thus, suggesting that the product of alternatively spliced PECAM-1 isoforms can be detected, which differ in their cytoplasmic domain.

To provide additional evidence for translation of PECAM-1 isoforms, we examined whether the mRNAs for alternatively spliced PECAM-1 isoforms are loaded on polysomes suggesting translation of the mRNA. Our cloning and sequencing data indicated that five different PECAM-1 isoforms mRNAs could be detected in the polysomes prepared from the HEL cells. These include the full-length PECAM-1, and PECAM-1 isoforms Δ12, Δ13, Δ14, and Δ15, consistent with the RT-PCR data. The detection of the various alternatively spliced PECAM-1

```
Full length      R K A K A K Q K P V E M S R P A V P L L N S N S E L V S
Δ12              R K A K A K Q K P V E M S R P A V P L L N S N S E L V S
Δ15              R K A K A K Q K P V E M S R P A V P L L N S N S E L V S
Δ14&15           R K A K A K Q K P V E M S R P A V P L L N S N S E L V S
Δ12,14&15        R K A K A K Q K P V E M S R P A V P L L N S N S E L V S

     E P S V E T T S H Y G Y D D V S E I D A V K P I N Q N K D S Q N M D V
     E P S V E T T S H Y - - - - - - - - - - - - - - - - - D S Q N M D V
     E P S V E T T S H Y G Y D D V S E I D A V K P I N Q N K D S Q N M D V
     E P S V E T T S H Y G Y D D V S E I D A V K P I N Q N K D S Q N M D V
     E P S V E T T S H Y - - - - - - - - - - - - - - - - - D S Q N M D V

     E Y T E V E V S S L E P H Q A L G T R A T E T V Y S E I R K V D P N F
     E Y T E V E V S S L E P H Q A L G T R A T E T V Y S E I R K V D P N F
     E Y T E V E V S S L E P H Q A L G T R A T E T V Y S E I R K V D P - -
     E Y T E V E V S S L E P H Q - - - - - - - - - - - - - - - - - - - -
     E Y T E V E V S S L E P H Q - - - - - - - - - - - - - - - - - - - -

     V G N R Y S R T E G L L D G T *
     V G N R Y S R T E G L L D G T *
     - - - - - - K N G R L P *
     - - - - - - E N G R L P *
     - - - - - - E N G R L P *
```

Fig. 8. The amino acid sequence of the rat PECAM-1 isoform cytoplasmic domains. In the amino acid sequences, the rat PECAM-1 isoforms lacking exon 12, 15, 14 and 15, or 12, 14 and 15 are compared to the rat putative full-length PECAM-1 isoform (Fig. 6). The deleted sequences are indicated by hyphens (-). The predicated exon sequences were adapted from human and murine PECAM-1, Newman et al. [1990] and Xie and Muller [1993], respectively. The underlined sequences indicate changes in the reading-frame of the amino acid sequence.
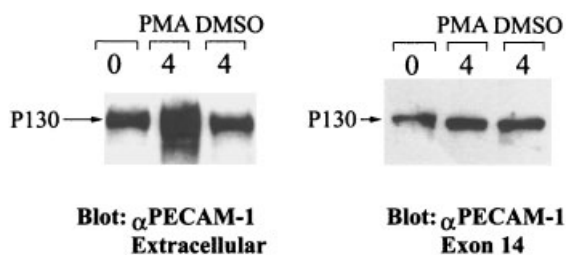
Fig. 9. Western blot analysis of PECAM-1 isoforms in U937 cells. Lysates were prepared from control (DMSO) and PMA-treated cells at designated time points (0 and 4 days) as described in Materials and Methods. Equal amounts of lysates (25 μg) from each sample were analyzed under reducing conditions utilizing SDS–PAGE. Proteins were transferred to Nitrocellulose membrane and blotted with the rabbit polyclonal antibody to extracellular domain (left panel) or exon 14 (right panel) of PECAM-1. Please note the presence of the lower molecular weight PECAM-1 bands in the 4-day PMA treated cells detected by the antibody to the extracellular domain of PECAM-1 but not the exon 14.

mRNAs, which were loaded onto the polysomes provided additional support for translation of PECAM-1 isoforms.

DISCUSSION

PECAM-1 (CD31) plays an important role during angiogenesis [DeLisser et al., 1997; Sheibani and Frazier, 1998, 1999; Cao et al., 2002] and transendothelial migration of leukocytes [Muller, 1995]. We have recently demonstrated that multiple isoforms of PECAM-1 are expressed in vascular beds of human and mouse tissue in a tissue and species specific manner [Sheibani et al., 1999; Wang et al., 2002], which can be developmentally regulated [Sheibani et al., 1999]. Hematopoietic cells express PECAM-1 on their surface and unlike EC does not take part in homophilic and/or heterophilic cell–cell interactions with the exception of transendothelial migration. In addition, antibody ligation of PECAM-1 on the surface of hematopoietic cells and platelets activates β1, β2, and β3 integrins [Tanaka et al., 1992; Piali et al., 1993; Leavesley et al., 1994; Berman and Muller, 1995; Berman et al., 1996; Chiba et al., 1999]. However, the identity of PECAM-1 isoforms and the downstream signaling events involved in these processes remain largely unknown. Alterations in the PECAM-1 cytoplasmic domain by alternative splicing, PECAM-1 phosphorylation, and changes in PECAM-1 expression levels are potential ways to modulate its adhesive properties [Yan et al., 1995; Sun

et al., 1996; Sheibani et al., 2000]. In order to further understand the function of PECAM-1 in hematopoietic cells and inflammation we determined the distribution of PECAM-1 isoforms in a variety of hematopoietic cells and platelets. Here, we demonstrate that: (1) hematopoietic cells and platelets express multiple isoforms of PECAM-1 in a lineage and species specific manner; (2) these patterns are very similar to the patterns of PECAM-1 isoforms detected in the endothelium; (3) the predominant isoform detected in human cells is the full-length PECAM-1, while in rodent cells Δ14&15 or Δ12,14&15 is the predominant isoform; (4) a novel PECAM-1 isoform Δ13&14 was detected in human hematopoietic cells, but is not present in endothelial cells; and (5) the product of the PECAM-1 isoforms is made and can be modulated in various lymphocytic cell lines.

The PECAM-1 isoforms are generated by alternative splicing of the exons encoding the PECAM-1 cytoplasmic domain (exons 10 through 16). The frequency at which the human PECAM-1 mRNA undergoes alternative splicing is much lower than that detected in rodent PECAM-1 mRNA in the endothelium [Sheibani et al., 1999; Wang et al., 2002]. This is consistent with the data presented here, that is rodent hematopoietic cells express more PECAM-1 isoforms than the human cells (Tables I, II, and III). This is perhaps, at least in part, due to greater stability of human genome compared to rodent genome. There are eight murine PECAM-1 isoforms and six human PECAM-1 isoforms identified in the endothelium [Sheibani et al., 1999; Wang et al., 2002]. Identification of PECAM-1 isoforms in hematopoietic cells will help to elucidate the role of PECAM-1 in hematopoiesis and modulation of their cellular adhesive properties.

We show here that multiple PECAM-1 isoforms are present in human and rodent hematopoietic cells. These included seven human PECAM isoforms, the full length, Δ12, Δ13, Δ13&14, Δ14, Δ15, and Δ14&15; six murine PECAM-1 isoforms, the full length, Δ14, Δ15, Δ12&14, Δ14&15, and Δ12, Δ14&15; and four rat PECAM-1 isoforms, Δ12, Δ15, Δ14&15, and Δ12,14&15. The isoform Δ13&14 is detected in human leukemia cells, but is not detected in rodent hematopoietic cells. In addition, full-length PECAM-1 is the predominant isoform detected in human cells (Table I), while the isoforms Δ14&15 or Δ12,14&15 is the predominant

isoform in the rodent cells. These patterns are very similar to those detected in human and murine endothelium [Sheibani et al., 1999; Wang et al., 2002]. The PECAM-1 isoform $\Delta15$ was detected at variable frequencies in all human cells whereas the isoforms $\Delta14\&15$ or $\Delta12,14\&15$ were detected in murine cells with relatively high frequencies. The isoform $\Delta15$ is the second predominant isoform in murine endothelium [Sheibani et al., 1999]. Mouse hematopoietic cells express this isoform at lower frequencies (Table II) compared to murine endothelium. Variations in the distributions of PECAM-1 isoforms occurred among the murine cells. In addition, human leukemia cell lines (HEL, U937, and Jurkat) showed more diversity in PECAM-1 pre-mRNA alternative splicing compared to human primary T cells and platelets (Table I). The immortalized human endothelial cells (THMVEC) also show more diversity in PECAM-1 pre-mRNA alternative splicing compared to primary endothelial cells [Wang et al., 2002]. The increased prevalence of PECAM-1 alternative splicing in THMVEC, HEL, U937, and Jurkat cells compared to primary EC, platelets, and primary T cells can be attributed, at least in part, to the rapid proliferating capacity and the unstable genome of these transformed cells.

To demonstrate that the products of different PECAM-1 isoforms are translated we generated an exon 14-peptide antibody, which discriminates among isoforms of PECAM-1 with and without exon 14. Using this antibody, we showed that not only the product of the murine PECAM-1 isoform(s) is present in the endothelium but also their expression is developmentally regulated [Sheibani et al., 1999]. We recently showed that different human PECAM-1 isoforms are also translated [Wang et al., 2002]. Here, we show that different alternatively spliced PECAM-1 isoforms are also translated in U937 cells using this antibody (Fig. 9). The major band detected in U937 cells corresponds to the 130 kDa full-length PECAM-1. A lower molecular weight band (110–120 kDa) is also present at very low levels, but it increases following incubation with PMA. The inability of exon 14 antibody to react with this band suggests the presence of isoform(s) without exon 14 and strongly argues against the possibility that the lower band is a glycosylation variant [Goldberger et al., 1994b] of full-length PECAM-1.

To further demonstrate that the PECAM-1 isoform(s) get translated we determined the identity of PECAM-1 isoform mRNAs that are loaded on the polysomes for translation. Our RT-PCR and cloning analysis detected a number of PECAM-1 isoforms on the polysomes prepared from HEL cells further conforming the potential of these isoforms for translation. This is consistent with our previous Western analysis of PECAM-1 isoforms in these cells. Furthermore, the lack of full-length PECAM-1 in mouse WEHI-3 cells and the C129 platelets further demonstrates that PECAM-1 isoforms, other than full-length PECAM-1, are translated and detected (Table II). Taken together, our results demonstrate that PECAM-1 isoforms are translated in the hematopoietic cells, as well as endothelial cells. The function of these isoforms in hematopoiesis and their role during transendothelial migration awaits further investigation.

PECAM-1 shows a high degree of homology in mammalian species during evolution implying potential similarity in function. However, its function may be differently regulated among different species. Expression levels of PECAM-1 may play an important role in modulation of PECAM-1 functions. The expression levels of PECAM-1 affect angiogenic characteristics of endothelial cells in vitro [Sheibani and Frazier, 1998]. Modulation of PECAM-1 levels in bEND cells by anti-sense transfection showed that lack of PECAM-1 prevents extensive tube formation on Matrigel, while some levels of PECAM-1 enhanced tube formation. In addition, very high levels of PECAM-1 appeared to reduce the tube forming ability of bEND cells on the Matrigel. Changes in PECAM-1 expression also occur during cell differentiation and/or activation of hematopoietic cells [Zehnder et al., 1992; Watt et al., 1993; Goldberger et al., 1994a]. Therefore, modulation of PECAM-1 levels may play a role in regulation of its cell adhesive properties.

The exonic inclusion/exclusion of PECAM-1 cytoplasmic domain, resulting in conformational changes and/or loss of phosphorylation sites, may be additional ways to modulate PECAM-1 functions. Yan et al. [1995] showed that expression of murine PECAM-1 isoforms that contain exon 14 results in heterophilic interactions in L-cells, while those that lack exon 14 participated in homophilic interactions. In addition, the phosphorylation state of exon 14 (Y 686) can similarly modulate PECAM-1

adhesive properties, such that when phospory-lated it mediates heterophilic interactions, while its mutation (Y to F) promotes homophilic interactions in L-cells [Famiglietti et al., 1997]. Interestingly, greater than 90% of PECAM-1 molecules expressed in the WEHI-3 cells and C129 platelets lack exon 14. The CTLL-2 cells and FVBN platelets also express a higher number of PECAM-1 molecules without exon 14 (58% in the CTLL-2 cells, 64% in the FVBN platelets). Thus, expression of PECAM-1 iso-forms that lack exon 14, at least in these cells, is not sufficient to promote homophilic interaction. These isoforms also fail to participate in heterophilic cell–cell interactions under resting conditions. The molecular mechanisms, which prevent PECAM-1 mediated homotypic and/or heterotypic interactions in hematopoietic cells remain largely unknown. We recently demon-strated that expression of murine PECAM-1 with and without exon 14 (Δ15 and Δ14&15, the two predominant isoforms in the endothelium) can differentially modulate cadherin mediated cell–cell interaction when expressed in the kidney epithelial MDCK cells [Sheibani et al., 2000]. This is mediated through the differential ability of these isoforms to activate MAPK/ ERKs pathway. Thus, PECAM-1 can actively modulate cadherin-mediated cell–cell interac-tion and its junctional localization depends on the formation of adherens junctions. Therefore, the lack of cadherin mediated cell–cell inter-actions in hematopoietic cells may, at least in part, contribute to differences in the adhesive properties of PECAM-1 isoforms observed in these two cell types. However, cell type specific expression of large negatively charged proteins, such as CD43 on hematopoietic cells, may exert anti-adhesive effects [Manjunath et al., 1995].

In summary, the differences in the expression pattern of PECAM-1 isoforms in human and rodents may indicate utilization of different mechanism(s) to modulate its adhesive func-tion. Characterization of the adhesive proper-ties of alternatively spliced PECAM-1 isoforms and identification of intracellular proteins which specifically interact with these isoforms will allow us to elucidate the role of PECAM-1 in hemostasis, inflammation, and angiogenesis.

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