response to interferon. On activation by ds RNA, 2-5A synthetase synthesizes 2′-5′ oligoadenylates, which in turn activate ribonuclease L [which is involved in mRNA and ribosomal RNA degradation (*23*)]. The 2-5A synthetase activity may also play an important role in the establishment of the antiviral state mediated by interferon (*10*). Northern (RNA) blot analysis was performed with a probe derived from a 2-5A synthetase cDNA clone (*24*) on polyadenylated RNA that had been isolated from HeLa cells (+*tat* or −*tat*) after a 16-hour incubation with lymphoblastoid interferon. This cDNA clone hybridizes to 1.5-, 1.7-, 2.5-, and 3.5-kb mRNAs in HeLa cells (*24, 25*). HeLa (−*tat*) cells expressed increased amounts of mRNA for 2-5A synthetase in response to interferon (Fig. 5, lanes 1 and 2). The interferon-mediated induction of these mRNAs in HeLa (+*tat*) cells (Fig. 5, lanes 3 and 4) was similar to that observed in HeLa (−*tat*) cells. As an internal control, actin mRNA was measured and found to be constant (Fig. 5). These results suggest that the interferon receptors have not undergone qualitative or quantitative changes in *tat*-expressing cells and that a general inhibition of the interferon response did not occur in these cells.

Our results show that in HIV-1–infected cells p68 kinase is downregulated. We propose that *tat* is responsible for mediating this downregulation, as interferon-induced p68 kinase levels were decreased in HeLa cells expressing a functional *tat*, as compared to control cells in which *tat* is absent or mutated. More mutant *tat* proteins need to be examined in order to establish a correlation between *tat*-mediated trans-activation and p68 kinase downregulation. The mechanism by which *tat* decreases p68 kinase levels may occur transcriptionally or posttranscriptionally.

The observation that *tat*-expressing cells are deficient in their ability to upregulate the expression of p68 kinase in response to interferon may have physiological implications for the replication cycle of HIV-1. Expression of *tat* is essential for productive viral infection. This may be due, in part, to the role of the gene product in countering the antiviral state that is mediated by the activation of interferon-inducible proteins. Downregulation of p68 kinase could provide a means for the virus to switch from latency to active replication.

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32. We thank C. Goyer for advice and comments on the manuscript, M. Agy for help with the HIV infections of CEM cells, M. Wambach for technical assistance, C. Rosen for providing HeLa cell lines expressing *tat* and *tat* expression vectors, B. Williams for the 2-5A synthetase cDNA clone, B. Felber and G. Pavlakis for the polyclonal antibody to *tat*, and L. Kedes for the actin cDNA clone. Supported by grants from the American Foundation for AIDS Research (000946-7-RGR: AmFAR/Drexel Burnham Lambert Research Grant) and Cancer Research Society (Montréal) to N.S., and by NIH grants AI22646 and RR00166 to M.G.K. N.S. is the recipient of a Medical Research Council Scientist award from the Medical Research Council of Canada; S.R. is a recipient of a studentship from Fonds de Recherche en Santé du Québec; N.T.P. is a recipient of a studentship from the Medical Research Council of Canada; and A.G.H. is a research fellow of the Centre Nationale de la Recherche Scientifique, France, of which the Unité d'Oncologie Virale is a research group (U.A. 1157).

25 September 1989; accepted 23 January 1990

PECAM-1 (CD31) Cloning and Relation to Adhesion Molecules of the Immunoglobulin Gene Superfamily

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An antibody to a platelet integral membrane glycoprotein was found to cross-react with the previously identified CD31 myelomonocytic differentiation antigen and with hec7, an endothelial cell protein that is enriched at intercellular junctions. This antibody identified a complementary DNA clone from an endothelial cell library. The 130-kilodalton translated sequence contained six extracellular immunoglobulin (Ig)-like domains and was most similar to the cell adhesion molecule (CAM) subgroup of the Ig superfamily. This is the only known member of the CAM family on platelets. Its cell surface distribution suggests participation in cellular recognition events.

HUMAN PLATELETS PARTICIPATE IN the normal hemostatic process and after vascular injury change from unreactive disks to adherent, pseudopod-containing spheres. Many platelet functions, including adhesion to extracellular matrix components, self-association (aggregation), and spreading (*1*), are reproduced by other cell types. The realization that several membrane glycoproteins thought to be "platelet-specific" are also on other cells (*2*) has allowed a number of molecular mechanisms for mediating cell surface interactions during hemostasis, differentiation and development, wound healing, and oncogenesis to be redefined.

Platelets and endothelial cells share a number of common membrane components (*3*), including several members of the integrin family of cytoadhesive receptors (*4*). To identify additional surface glycoproteins that might participate in platelet and endothelial cell function, we prepared a polyclonal antibody to human platelet integral membrane proteins (*5*) and used this antibody to screen an endothelial cell λgt11 expression library. Antibody-positive clones

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were determined by "epitope selection" (*6*). Two clones, 8B and 8C, encoded fusion proteins that selected antibodies reactive on SDS–polyacrylamide gel electrophoresis (SDS-PAGE) immunoblots with a distinct 130-kD platelet–endothelial cell protein that we have designated PECAM-1 (platelet–endothelial cell adhesion molecule–1).

A panel of monoclonal antibodies to cell surface glycoproteins of similar size and *p*I were tested for their ability to react with PECAM-1. Two monoclonal antibodies (MAbs), SG134 and hec7, reacted strongly with a platelet protein that comigrated on SDS-polyacrylamide gels with PECAM-1. The SG134 MAb (*7*) is one of several International Workshop antibodies that recognize the CD31 antigen, a 130- to 140-kD glycoprotein that is expressed during differentiation of myelomonocytic cells (*8*). The hec7 MAb was produced in response to human umbilical vein endothelial cells, and reacts with a 135-kD membrane protein that is enriched at the intercellular junctions of continuous endothelia in all blood vessels (*9*, cover photograph). To examine the relation of these three proteins, the hec7 and SG134 antigens were immunoprecipitated from human platelet lysates with their respective MAbs and were centrifuged. Both the immunoprecipitated pellets and the residual proteins in the supernatants were separated by SDS-PAGE and transferred to an Immobilon membrane (Fig. 1). A rabbit antiserum to the PECAM-1 cDNA clone 8B fusion protein (anti–PECAM-1) detected PECAM-1 only in the specific immunoprecipitates; all PECAM-1–like material was removed from the platelet lysates by the MAbs to either hec7 or CD31. In additional experiments, hec7 or CD31 antigens were purified from endothelial cells, transferred to a membrane, and detected with the anti–PECAM-1 serum (*10*). Immunopurified PECAM-1 (*11*) was also recognized by the hec7 and CD31 MAbs. Together, these data integrate several disparate fields of investigation, and demonstrate that PECAM-1 is immunologically related or identical to the CD31 and hec7 antigens.

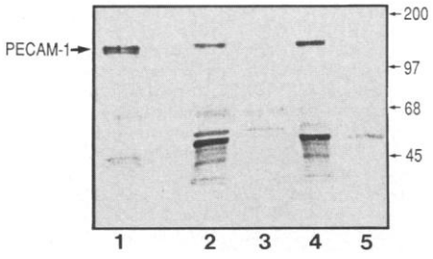

Fig. 1. PECAM-1 is related or identical to CD31 and hec7 antigens. The hec7 and CD31 antigens (lanes 2 and 4, respectively) were immunoprecipitated from human whole platelet detergent lysates (lane 1) with either the hec7 or SG134 (anti-CD31) murine MAbs. Samples were incubated with protein A–Sepharose beads that had been bound with goat antibody to mouse Ig and then centrifuged. The protein contents of both the pellets (lanes 2 and 4) and supernatants (lanes 3 and 5) were separated by SDS-PAGE under reducing conditions and transferred to an Immobilon membrane. Immunoblots were analyzed with anti–PECAM-1, a rabbit serum to the λgt11 fusion protein encoded by the PECAM-1 cDNA clone 8B. Binding of anti–PECAM-1 was detected as in (*6*). Molecular size marker positions (in kilodaltons) are indicated at the far right. Bands at 50 kD represent murine and goat IgG that cross-reacted with the anti–rabbit IgG detection system.

We established the complete nucleotide sequence of the coding region of PECAM-1 from three overlapping clones. The 2557-bp sequence (*12*) contains a 141-bp 5′ untranslated region, an open reading frame of 2214 bp that encodes 738 amino acids, and a 202-bp 3′ untranslated region. Hydropathicity analysis (*13*) of the deduced 738–amino acid sequence revealed the presence of two putative transmembrane domains (underlined in Fig. 2): the first within a putative signal sequence near the NH_2-terminus, and the second spanning residues 575 to 593 of the mature glycoprotein. We have assigned amino acid 27 (counting from the initiator Met) as the probable site of cleavage of the signal peptide from mature PECAM-1. This site conforms well with the [−3, −1] rule (*14*), and the assignment of the +1 Gln is also consistent with our inability to obtain NH_2-terminal sequence information from multiple purified protein preparations (*15*). The predicted size of the resulting mature 711-residue polypeptide chain is ~80 kD, with nine predicted asparagine-linked glycosylation sites (*16*) distributed over the molecule. The fully processed molecule is 130 kD on an immunoblot; thus carbohydrate residues must account for ~40% of the molecular size of PECAM-1. The orientation of PECAM-1 within the plasma membrane appears typical; the NH_2-terminal 574 amino acids is followed by a single 19-residue membrane-spanning domain and a 118-residue COOH-terminal cytoplasmic domain. The cytoplasmic domain contains a Tyr (marked with a ●) that could serve as a phosphorylation site for tyrosine kinase (*17*). All of the predicted NH_2-linked carbohydrates are external to the membrane, con-

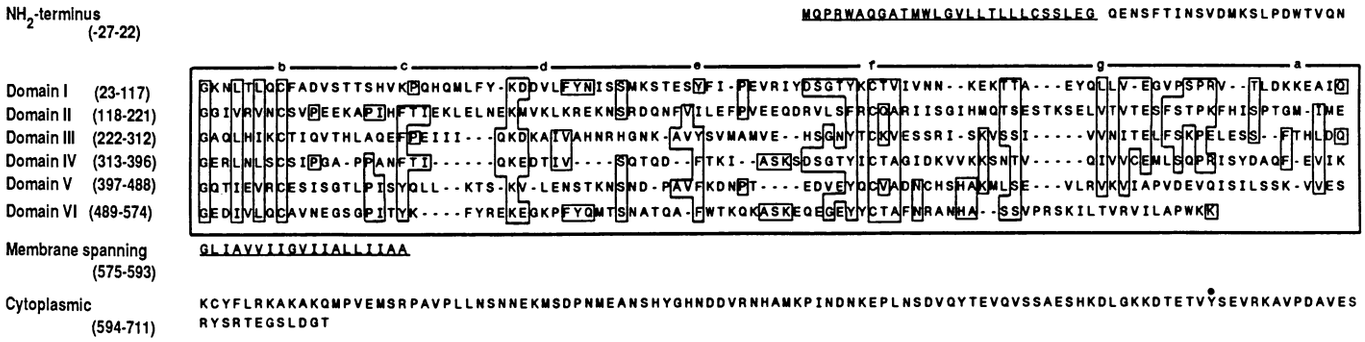

Fig. 2. Primary structure and alignment of the internal repeats of PECAM-1. The amino acid sequence is numbered from the first residue after the predicted cleavage site of the 27–amino acid signal peptide. The putative signal peptide and transmembrane sequences are underlined. The sequence contains nine N-linked glycosylation sites and one potential tyrosine phosphorylation site at Tyr^{686} (denoted with a closed circle). The six Ig type domains (numbered I–VI) contain 94, 103, 90, 93, 91, and 81 amino acids. Each domain contains two cysteine residues that are flanked by conserved amino acid residues typical of Ig type C2 domains. Sequences were aligned visually and with protein analysis programs. Dashes denote gaps that were introduced to maintain maximal alignment. Boxed amino acids correspond to residues shared by two or more PECAM-1 domains. The location of the predicted β-strands a to g are shown in lowercase letters above the alignments. PECAM-1 domains, like those of ICAM-1, lack the characteristically positioned Trp residues normally found within β-strand c of the Ig-fold. The cDNA insert from λgt11 clone 8B was subcloned into the pUC18-derived plasmid vector pTZ18r. Using the 8B insert as a probe, 23 additional clones were identified and subsequently characterized by restriction mapping and hybridization analysis. The complete amino acid sequence was determined from three overlapping phage clones. A series of overlapping nested deletions were generated using exonuclease III. Following exonuclease digestion, the remaining single-stranded material was removed by S1 nuclease, the ends blunted with T4 DNA polymerase, and the plasmids resealed with T4 DNA ligase. Both strands of all overlapping constructs were sequenced by the dideoxy chain termination method using the Sequenase™ DNA polymerase.

sistent with the reduction in molecular size of PECAM-1 from neuraminadase-treated platelets (*10*).

Twelve Cys residues are spaced approximately 50 amino acids apart throughout the external domain. Six immunoglobulin (Ig)-like homology units flank these cysteines; each unit contains the conserved pattern

Val Val
GlyXXLeuXLeuX**Cys**–35 to 55 residues–
Ile Ile

Val
AspXGlyXTyrX**Cys**XAla

that is characteristic of the Ig gene superfamily (*18*). Alignment of the six Ig-like domains with each other (Fig. 2) revealed intradomain sequence similarity, suggesting that this molecule arose by duplication of a single Ig-like homology unit. Williams has classified Ig domains into C1, C2, and V subgroups on the basis of the placement of distinctive highly conserved residues and predicted folding patterns (*18*). Molecules involved in antigen recognition and presentation are largely composed of C1 and V sets, whereas the C2 subgroup is predominantly found in the cell adhesion molecule (CAM) subfamily of Ig-like proteins. The Ig homology units in PECAM-1 are C2 domain structures as found in other members of the CAM family, including carcinoembryonic antigen (CEA), a homotypic intercellular adhesion molecule (*19*), ICAM-1, a surface membrane glycoprotein that promotes lymphocyte adhesion and inflammatory reactions (*20*), and the neuronal cell adhesion molecules of N-CAM, L1, fasciclin II, amalgam, and contactin (*21*). The C2 domains of the Ig gene superfamily are comprised of seven anti-parallel β strands arranged into two β-sheets that form the characteristic sandwich of the Ig-fold (*22*). Secondary structure analysis (*23*) of PECAM-1 revealed alternating groups of hydrophobic and hydrophilic amino acids, forming all seven expected β strands (labeled a to g in Fig. 2) in five of the six domains, with conserved cysteine residues in strands b and f forming the disulfide bridge that stabilizes the β-sheet sandwich. Domain 5 is incomplete, not having β strands a, b, d, and e that form one side of the sandwich. However, the other half of the Ig-fold (strands c, f, and g) are normal with respect to amino acid composition and predicted secondary structure. Since the functional significance of the Ig homology unit is unknown, the consequences of domain 5's unusual structure are difficult to predict.

Computer-assisted amino acid similarity searches (*24*) were used to determine relationships between PECAM-1 and the CAM family (Table 1 and Fig. 3). The sequences with the highest overall degree of homology included carcinoembryonic antigen, N-CAM, the c-*fms* protooncogene [equivalent to the colony-stimulating factor–1 receptor (*25*)], and ICAM-1. The greatest conservation was within the C2 domains surrounding the conserved cysteines responsible for stabilizing the Ig-fold. In addition to homology within the Ig domains themselves, PECAM-1 is similar to the c-*fms* protooncogene and the platelet-derived growth factor receptor (PDGFR) at their NH_2-termini. PECAM-1 and the PDGFR also share 32% identity in the tail region of their cytoplasmic domains. This region of the PDGFR may be involved in signal transduction, as antibodies to this region selectively recognize a conformation of the receptor present only when occupied by its ligand, PDGF (*26*). Whether or not this region in PECAM-1 also mediates ligand-induced signal transduction remains to be determined.

No other member of the CAM family has been described on human platelets. A role for PECAM-1 in cell-cell recognition or adhesion is suggested by its distribution on the surface of human platelets and endothelial cells, its localization to endothelial cell intercellular junctions [cover photo (*8, 9*)], by its primary and predicted secondary structure, and by its homology with other members of the CAM family of cytoadhesive receptors. Perhaps exposure of junctional PECAM-1 at sites of vascular injury could serve as an adhesive triggering mechanism for subsequent platelet adhesion and formation of the platelet plug. Exposure of this molecule within the platelet thrombus may also provide a surface to which monocytes and granulocytes, which also express PECAM-1 (*7, 8*), can adhere. This potential "homing activity" could initiate the inflammation and wound-healing process.

Finally, several closely related members of the Ig superfamily can serve as receptors for some clinically important viruses (*27*). Thus, the major rhinovirus receptor is ICAM-1, the receptor for human immunodeficiency virus is CD4, and the poliovirus receptor is a cell surface glycoprotein containing three Ig-like loop domains. Although it is clearly not the function, but rather the misfortune, of these Ig-like molecules to act as viral receptors, the possibility remains that other glycoproteins comprised of Ig homology units may also serve as a means for viruses to escape normal host immune surveillance, leading to enhanced viral survival and subsequent infection. Whether PECAM-1 participates in the entry of viruses into platelets and endothelial cells remains an intriguing avenue of future investigation.

Table 1. Homology of PECAM-1 with other Ig-superfamily members. Sequence comparisons were performed using the FASTP program originally developed by Lipman and Pearson (*24*) and adapted for the PC-based program, CD-GENE. Optimized scores were obtained from the highest scoring initial sequences by inserting gaps and recalculating maximal alignments; AA, amino acids.

Protein	Optimized score	Nature of homology
Carcinoembryonic antigen	191	21.3% identity in 343 AA overlap
N-CAM	119	15.2% identity in 328 AA overlap
Murine Fc receptor	98	27.3% identity in 128 AA overlap
CSF receptor (c-*fms*)	82	15.6% identity in 378 AA overlap
ICAM-1	81	25.6% identity in 82 AA overlap
PDGFR	80	18.0% identity in 133 AA overlap
CD4	59	24.7% identity in 85 AA overlap
Poliovirus receptor	50	28.8% identity in 59 AA overlap

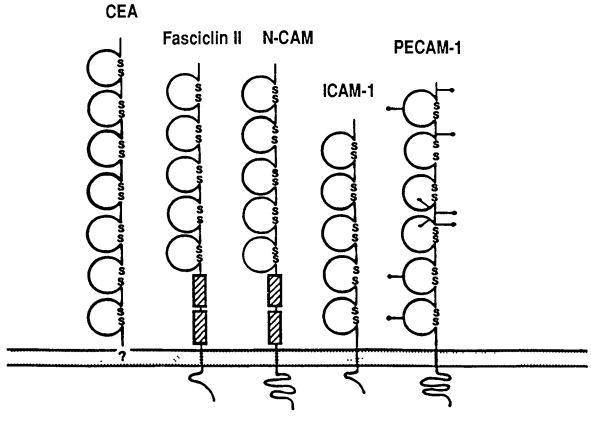

Fig. 3. Domain structure of PECAM-1 and related CAM members of the Ig gene superfamily. The circles are representations of individual Ig homology units composed of two antiparallel β-sheets. All of the CAMs shown are made up of C2-type domains, with the exception of carcinoembryonic antigen (CEA), which has one V-type domain at the NH_2-terminus. All NH_2-termini are extracellular. The hatched boxes proximal to the membrane in fasciclin II and N-CAM represent fibronectin type III domains. Potential N-linked carbohydrate chains on PECAM-1 are symbolized by a (——●).

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31. We thank C.-J. Edgell and D. Ginsberg for providing endothelial cell cDNA libraries and S. Goyert for providing the anti-CD31 International Workshop antibody, SG134. W.A.M. is the recipient of a Pew Scholarship and an RJR-Nabisco Research Scholars Award. Supported by grant HL-40926 (to P.J.N.) from the National Institutes of Health.

2 October 1989; accepted 8 January 1990

Ribozymes as Potential Anti–HIV-1 Therapeutic Agents

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Certain RNA molecules, called ribozymes, possess enzymatic, self-cleaving activity. The cleavage reaction is catalytic and no energy source is required. Ribozymes of the "hammerhead" motif were identified in plant RNA pathogens. These ribozymes possess unique secondary (and possibly tertiary) structures critical for their cleavage ability. The present study shows precise cleavage of human immunodeficiency virus type 1 (HIV-1) sequences in a cell-free system by hammerhead ribozymes. In addition to the cell-free studies, human cells stably expressing a hammerhead ribozyme targeted to HIV-1 *gag* transcripts have been constructed. When these cells were challenged with HIV-1, a substantial reduction in the level of HIV-1 *gag* RNA relative to that in nonribozyme-expressing cells, was observed. The reduction in *gag* RNA was reflected in a reduction in antigen p24 levels. These results suggest the feasibility of developing ribozymes as therapeutic agents against human pathogens such as HIV-1.

INVESTIGATORS HAVE SHOWN THE ability of antisense RNAs to impair gene expression (*1*) and have suggested the use of these molecules as antiviral and anticancer agents. However, there are several limitations to this approach imposed, in part, by the stoichiometric nature of the inhibition. Alternatively, the observation that certain RNA molecules (ribozymes) possess enzymatic, self-cleaving activity (*2–5*) suggests that antisense molecules could be developed that not only form RNA-RNA hybrids, but also catalytically cleave a phosphodiester bond in the target RNA strand. Because the catalytic RNA would not be consumed during the cleavage reaction, a large number of substrate molecules could be processed (Fig. 1). Two structural motifs, hammerhead (*5, 6*) and hairpin (*7*), have thus far been described as intermediates in these self-cleavage reactions.

Ribozymes in the hammerhead family share a high degree of similarity in primary and secondary structure (*6, 8, 9*). The hammerhead consists of three stems and a catalytic center containing 13 conserved nucleotides (5′GAAAC$(N)_n$GUN$(N)_n$CUGA(N)GA3′) (Fig. 2). Natural catalytic centers may be formed by contiguous regions in the RNA (*6, 8*) or by regions separated by a large number of nucleotides (*5, 10*). Cleavage occurs 3′ to the GUX triplet where X can be C, U, or A (*11–13*), generating 2′,3′-cyclic phosphate and 5′ hydroxyl termini (*4, 5*). The essential constituents for the hammerhead can be on separate molecules, with one strand serving as a catalyst and the other as a substrate (*11, 12*) (Fig. 1). Further,

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