MINIREVIEW

PADGEM: An Adhesion Receptor for Leukocytes on Stimulated Platelets and Endothelial Cells (43309A)

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Platelets are anucleate cells that circulate in the blood in a resting form. At the site of tissue injury, platelets undergo a variety of morphological and biochemical modifications. These include a shape change with the formation of pseudopodia (1); the secretion of the content of storage granules, namely the α-granules, the dense granules, and the lysosomes (2); and the expression of new receptors (3) and of procoagulant activity (4) on the cell surface. *In vitro*, this activation process can be triggered by numerous agonists, such as thrombin, collagen, epinephrine, ADP, and the calcium ionophore A23187 (5). Upon activation, platelets also shed membrane microparticles rich in procoagulant activity (6).

Using a monoclonal antibody, KC4, raised against activated platelets that does not bind to unstimulated platelets, we identified a novel protein that is expressed on the surface of platelets only after their activation (7). This protein was designated platelet activation-dependent granule to external membrane protein (PADGEM) (8).

Using a similar approach and a monoclonal antibody called S12, McEver and Martin (9) identified an antigen expressed on the surface of platelets only after their activation; although originally thought to be glycoprotein (GP) IIa, this antigen was subsequently proven to be a novel protein, since termed granule membrane protein 140 (10). Cooperative studies led to the conclusion that PADGEM and GMP 140 are the same protein. This protein has also been given the designations LECAM 3 (11) and CD62 (12); recently we and our colleagues have agreed to a new nomenclature in which this protein has been renamed P-selectin.

Subcellular Localization and Cell Distribution of PADGEM

Immunofluorescence staining of resting platelets with KC4 is negative, but permeabilized platelets show a punctate pattern of fluorescence in the cell interior. Thrombin-stimulated platelets that are not permeabilized stain with a rim pattern, demonstrating the translocation of PADGEM from an internal location to the cell surface upon activation (8). By immunoelectron microscopy, organelle fractionation, and differential granule release, PADGEM was found to be a component of the α-granule of unstimulated platelets (8). PADGEM meets the chemical criteria of an integral membrane protein and is, therefore, considered an intrinsic component of the α-granule membrane. Upon platelet activation and α-granule secretion concomitant with the fusion of the granule membrane with the plasma membrane, this protein is translocated to the external membrane (Fig. 1) (8). Activated platelets express on their surface approximately 13,000 copies of PADGEM per cell, while resting platelets have none (7). This protein is also present in platelet-derived microparticles (13). Platelets from patients with the rare disorder gray platelet syndrome have a deficiency of α-granules (14). These platelets exhibit internal membrane-bound PADGEM that is translocated to the external membrane upon platelet activation (15).

PADGEM had been thought to be unique to plate-

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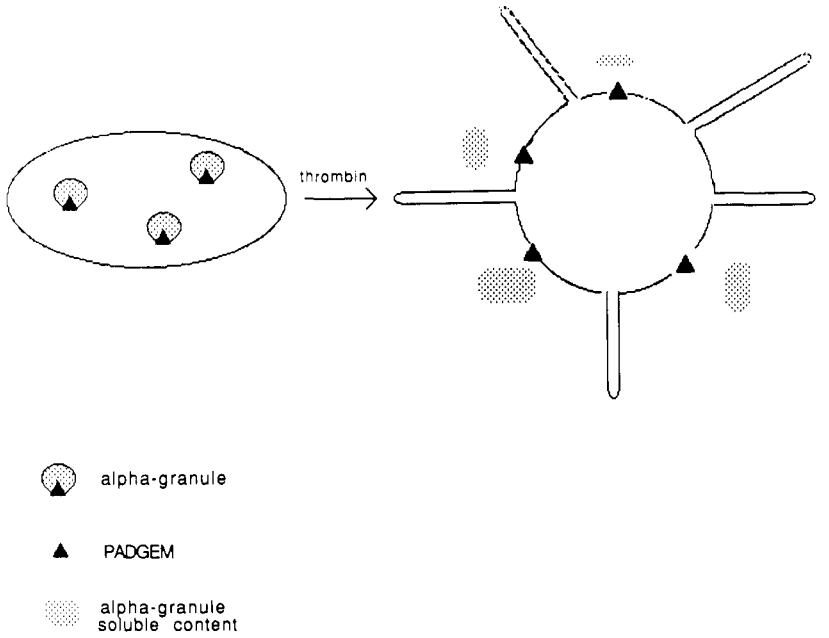

Figure 1. Schematic representation of the translocation of PADGEM from the α-granule membrane to the external membrane upon platelet activation.

lets and their precursors, megakaryocytes (16). However, its presence has been more recently demonstrated in endothelial cells (17, 18) and in the human erythroleukemia cell line that expresses markers of megakaryocytic origin (19). Although human erythroleukemia cell lines contain α-granule-like organelles and α-granule-associated proteins, immunofluorescence staining of human erythroleukemia cell lines with anti-PADGEM antibodies demonstrates the expression of PADGEM only on the cell surface (19). In contrast, when anti-PADGEM antibodies are used to stain endothelial cells, rod-shaped, elongated organelles are visualized; upon stimulation with the calcium ionophore A23187 and thrombin, these vesicles disappear (17). Double immunofluorescence staining with antibodies against PADGEM and von Willebrand factor showed that these granules are the Weibel-Palade bodies (17).

The Structure of PADGEM

PADGEM migrates in sodium dodecyl sulfate polyacrylamide gel electrophoresis with an apparent mol wt of 140,000 under nonreducing conditions; upon reduction, its migration is slightly slower, suggesting the presence of numerous intrachain disulfide bonds (7). Carbohydrate analysis of PADGEM revealed the presence of sialic acid, neutral sugars, and glucosamine residues, which totaled 28.8% by weight of the glycoprotein (20).

PADGEM was cloned from a human umbilical vein endothelial cell cDNA library in λgt11 (21). The cDNA-derived amino acid sequence predicted a characteristic domain structure. Starting at the N terminus, after a cleavable signal peptide (residues −41 to −1), the first domain of the mature protein (residues 1–118) shares homology with several carbohydrate-binding proteins and is called the "lectin" domain. The second domain (residues 119–158) is similar to the epidermal growth factor (EGF) precursor and the EGF-like domain in a number of proteins, including the coagulation Factors VII, IX, X, and XII, protein C, and protein S (22). Starting at residue 120, a series of nine tandem repeats begins, each composed of about 62 residues, including six conserved cysteines. These repeats are homologous with complement-binding proteins, as well as complement regulatory proteins, and are referred to as "complement" repeats. A putative transmembrane domain begins at residue 731 and is followed by a short cytoplasmic tail (residues 775–789) (21). Twelve potential asparagine-linked glycosylation sites are present (21).

One in-frame deletion was identified in some PADGEM cDNA clones. The consequence of this deletion is the removal of the putative transmembrane domain (21); this finding suggests that a soluble form of PADGEM might be synthesized, but this molecule has not been fully characterized at the protein level to date.

The cDNA sequence that we obtained in our laboratory is identical to the published sequence, except for five nucleotides within the coding sequence. Only two of these base changes alter the predicted amino acid sequence (proline instead of serine at position −21 and threonine instead of isoleucine at position 233) (23).

The predicted domain structure of PADGEM shows a high degree of similarity with that of two

recently characterized molecules: ELAM-1 (24) and the mouse lymphocyte homing receptor MEL14 (or $gp90^{mel}$) (25, 26). Both these proteins have a lectin domain followed by an EGF domain, a variable number of complement repeats (six in ELAM-1 and two in MEL14), a transmembrane domain, and a cytoplasmic tail. ELAM-1 is synthesized by cytokine-stimulated endothelial cells and functions as an adhesion receptor mediating the binding of stimulated endothelium to neutrophils (24). MEL14 is also an adhesion receptor; it is found on the surface of circulating lymphocytes and binds to high endothelial venules in peripheral lymph nodes (25, 26). More recently, a human analog of MEL14, LAM-1, has been characterized (27). Northern analysis showed the presence of LAM-1 mRNA in T lymphocytes and some human B cell lines (27).

The Function of PADGEM

Based on the structural homology of PADGEM with these adhesion molecules, we evaluated the potential role of PADGEM as an adhesion receptor on the surface of activated platelets. Activated, but not resting, platelets bind *in vitro* to blood monocytes and neutrophils, as well as to the human leukemia cell lines with "monocytoid" characteristics, U937 and HL60 (28). This interaction requires the presence of Ca^{2+} ions. Polyclonal anti-PADGEM antibodies, as well as purified PADGEM, totally inhibit this interaction (29). Thrombospondin, albumin, the tetrapeptide Arg-Gly-Asp-Ser, anti-GPIIb/IIIa, anti-thrombospondin, and anti-GPIV antibodies have no effect. When PADGEM is incorporated into phospholipid vesicles to prevent the nonspecific interaction of its transmembrane domain with cell membranes, a saturable binding of PADGEM to U937 cells is observed. The binding is specific and does not occur with Jurkat cells, another human leukemia cell line that does not bind to activated platelets. Besides U937 cells, PADGEM-phospholipid vesicles also bind to fresh monocytes, neutrophils, and HL60 cells, and not to red blood cells or lymphocytes (29).

These observations have been extended to endothelial cell PADGEM (30). Phorbol myristate acetate- or histamine-stimulated endothelial cells develop the ability to bind neutrophils within minutes; this activity does not require protein synthesis. Antibodies to PADGEM inhibit the binding of neutrophils to stimulated endothelium (30).

These data support the hypothesis that PADGEM is indeed an adhesion receptor that mediates the interaction of stimulated platelets and endothelial cells with monocytes and neutrophils. Therefore, this glycoprotein shares functional, as well as structural, homology with ELAM-1 and MEL14/LAM-1. PADGEM, ELAM-1, MEL14, and LAM-1 are now considered members of a new family of adhesion molecules called LECCAMs (31) or selectins (32). A scheme of the structure of the members of this family of proteins is shown in Figure 2.

The Ligand for PADGEM on Leukocytes

In order to characterize the natural ligand for PADGEM on leukocytes, a panel of monoclonal antibodies was tested in our laboratory to identify an antibody that, bound to the cells expressing the PADGEM recognition site, did not bind to platelets and inhibited the adhesion of activated platelets to leukocytes. Three different antibodies of the CD15 cluster met the above criteria (23). The antigen recognized by the CD15 antibodies is an oligosaccharide associated with glycolipids, glycoproteins, and proteoglycans and is called lacto-*N*-fucopentaose (LNF) III (33, 34). This carbohydrate has the structure Galβ1-4 (Fucα1-3) GlcNacβ1-3Galβ1-4Glc; its postitional isomers LNF I and LNF II are not recognized by the CD15 antibodies. The core component of LNF III is Galβ1-4 (Fucα1-3) GlcNAc, also known as Lewis X (Le^x). Figure 3 shows the structure of these carbohydrates.

The CD15 antigen LNF III also inhibits the adhesion of activated platelets to neutrophils. A 50% inhibition is obtained with a concentration of LNF III of about 250 μM. LNF II has a small inhibitory effect that could be due to LNF III contamination, whereas LNF I has none (23).

Le^x is present on the surface of neutrophils and monocytes. This carbohydrate is present in several leukocyte glycoproteins, including LFA-1, Mac-1, CR1, the complex CD11/CD18, a phosphotyrosine-containing protein with a mol wt of about 180,000, and two other proteins with mol wt of 205,000 and 145,000. Although these proteins are distributed among other cells types, Le^x is part of their structure only in monocytes and neutrophils (34, 35).

Taken as a whole, the above data indicate that Le^x is a component of the PADGEM ligand. A recent observation that neuraminidase decreases the interaction of activated platelets with treated HL60 cells emphasizes that a terminal sialic acid is also a component of the PADGEM ligand (36). The ELAM-1 ligand, Sialyl Le^x (37–39), is structurally related to the PADGEM ligand, but recent unpublished data emphasize structural differences in the ligands for the two selectins.

Future Directions

The physiologic role of PADGEM remains speculative. Activated platelets are thrombogenic and must be removed from the circulation. PADGEM-mediated binding of activated platelets to the monocyte-macrophages in the reticuloendothelial system might represent a means of clearing these cells from the bloodstream. Similar considerations apply to platelet-derived microparticles.

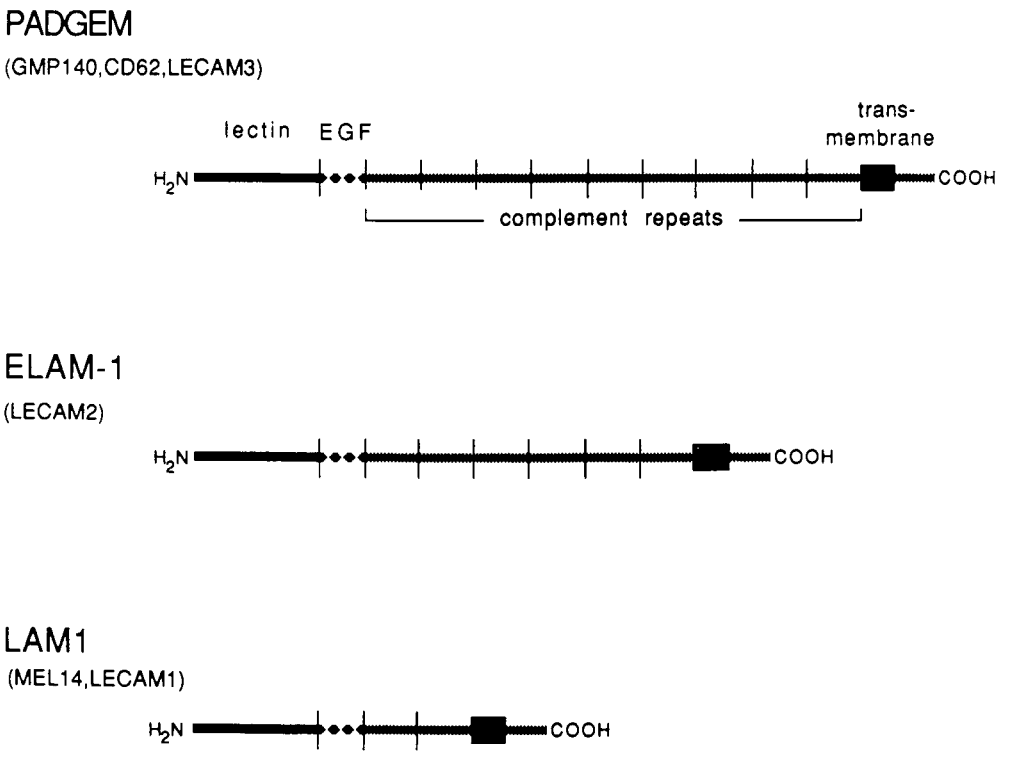

Figure 2. Domain structure of the members of the selectin family.

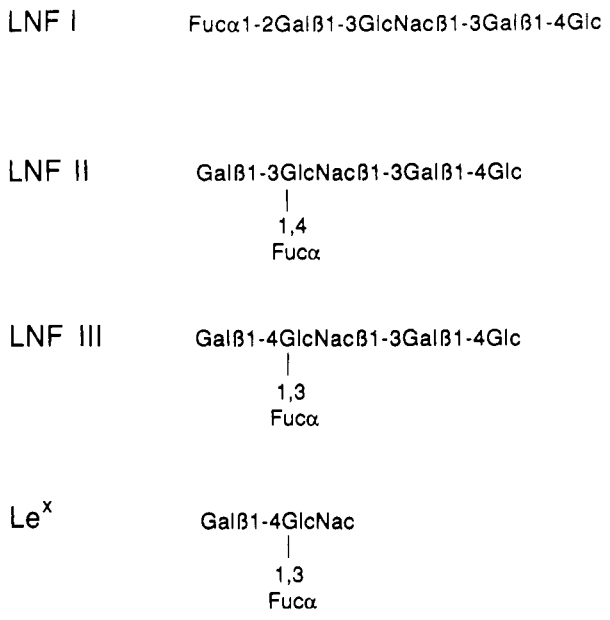

Figure 3. Structure of lacto-*N*-fucopentaoses and of Lewis X.

The expression of platelet PADGEM could also contribute to the assembly of cellular components necessary for fibrin formation. The monocyte is the only circulating blood cell capable of expressing tissue factor, a co-factor for coagulation Factor VII that is required for the initiation of the extrinsic coagulation pathway. Monocytes may be recruited by activated platelets through a PADGEM-mediated mechanism to the site of tissue injury.

Stimulation of the endothelium with inflammatory effectors, such as histamine, leads to the rapid degranulation of the Weibel-Palade bodies and the expression of PADGEM, which mediates the recruitment of neutrophils and monocytes; these cells are, in turn, capable of amplifying the inflammatory response (30). PADGEM also inhibits the adhesion of cytokine-stimulated neutrophils to unstimulated endothelial cells, a phenomenon mediated by the CD18 complex on the leukocytes (40). It has been proposed that the secretion of a soluble form of PADGEM might limit this adhesion phenomenon (40). PADGEM might, therefore, play a major role in the regulation of inflammatory processes.

Whatever the physiologic role(s) of PADGEM might be, this molecule can be used as a marker of platelet activation *in vivo*. Since activated platelets are concentrated at the site of thrombus formation, the use of radiolabeled antibodies to PADGEM to image thrombi has been proposed (41). Using this approach, deep venous thrombi were visualized in a baboon model within 10 min with no subtraction techniques, and the image was optimal within 1 hr (Fig. 4); the target to blood pool tracer ratio was over 3:1. Radioimmunoscintigraphy with anti-PADGEM antibodies is, therefore, a promising technique for the detection of thrombi in humans (41). Anti-PADGEM antibodies were also used to evaluate the extent of platelet activation *ex vivo* in patients with coronary artery disease. Preliminary reports show that the percentage of platelets expressing PADGEM on their surface is higher in

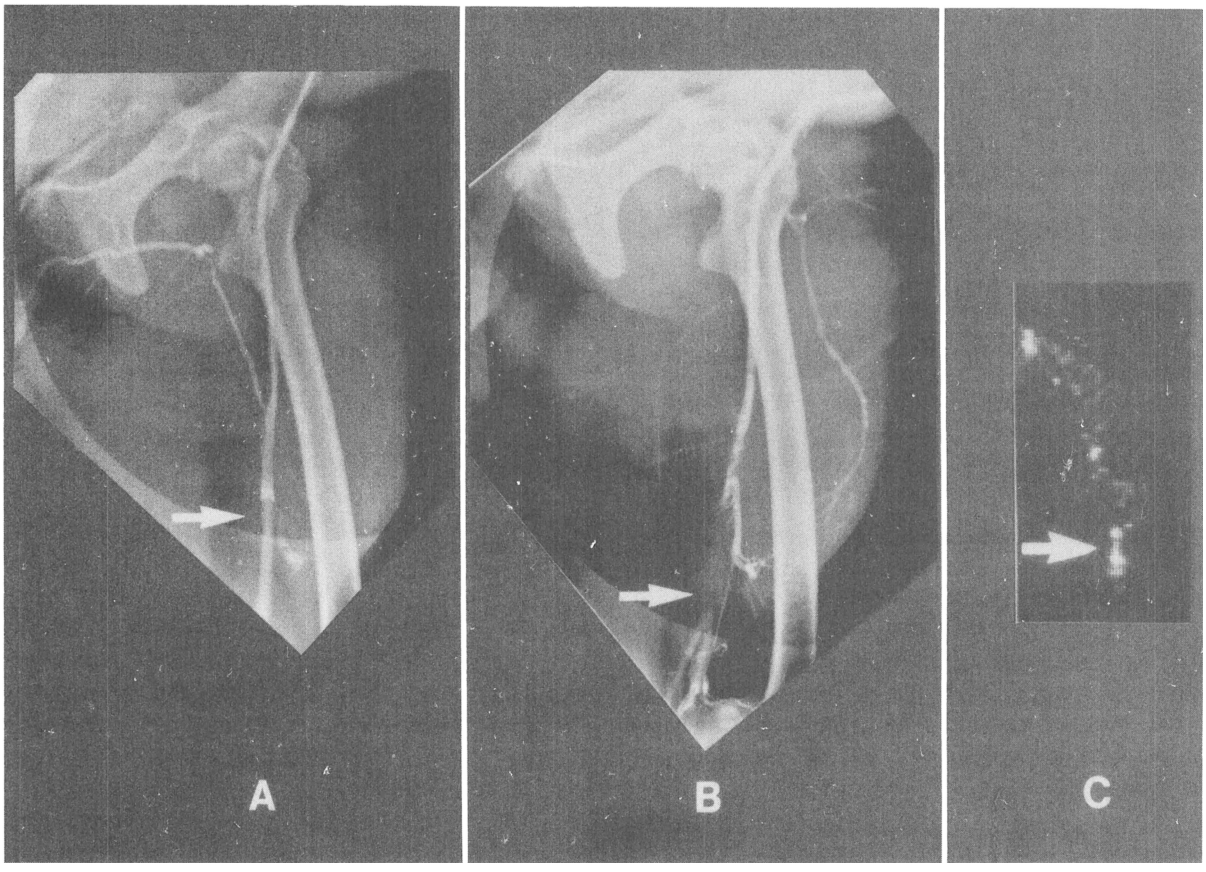

Figure 4. Venogram and immunoscintigraphy of a deep vein thrombus in a baboon model with ^{123}I anti-PADGEM antibody. (A) Venogram of the left femoral vein prior to thrombus formation. (B) Venogram of the same vein after formation of a thrombus (arrow). (C) Scintigram of the same thrombus obtained 60 min after infusion of ^{123}I anti-PADGEM antibody. From Ref. 41.

patients with unstable angina than in normal patients (25% vs 2% in the peripheral vein blood); patients with stable angina had an intermediate level of platelet activation (6%) (42). An increase in the percentage of PADGEM-positive platelets was also observed after percutaneous transluminal coronary angioplasty. This increase was much more evident in four patients who developed vessel closure and/or acute myocardial infarction (43). The assessment of PADGEM expression by circulating platelets may shed light on the role of platelet activation in the pathophysiology of coronary artery disease and in the platelet response to pharmacological interventions. Also, blockage of the PADGEM-mediated adhesive reactions could reduce inflammatory and thrombotic phenomena in these patients.

The binding of tumor cells to platelets has long been recognized as a potentially important mechanism of metastasis generation due to the formation of tumor-platelet emboli (44). More recently, a role for numerous platelet adhesive proteins, including fibronectin, von Willebrand factor, and the glycoprotein complex GPIIb/IIIa, in platelet-tumor cell interaction and in metastasis formation *in vivo* has been demonstrated (45, 46). Le^x, a part of the receptor for PADGEM on leukocytes, is a tumor-associated antigen (47). Therefore, PADGEM could be involved in the binding of platelets to certain tumor cells. Furthermore, PADGEM expressed on the surface of endothelial cells could function as an adhesion receptor for the same tumor cells, allowing them to metastasize. Reagents that block PADGEM-tumor cell interaction might prove useful in limiting the diffusion of metastases via this mechanism.

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