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GMP-140: A Receptor for Neutrophils and Monocytes on Activated Platelets and Endothelium

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Abstract GMP-140 is a membrane glycoprotein located in secretory granules of platelets and endothelium. When these cells are activated by agonists such as thrombin, GMP-140 is rapidly translocated to the plasma membrane. GMP-140, along with ELAM-1 and the peripheral lymph node homing receptor, defines the selectin family of structurally related molecules that regulate interactions of leukocytes with the blood vessel wall. Each of these molecules contains an N-terminal lectin-like domain, followed by an EGF-like region, a series of consensus repeats related to those in complement-binding proteins, a transmembrane domain, and a short cytoplasmic tail. The genomic structures of the selectins suggest that they arose by duplication and modification of exons encoding specific structural domains. GMP-140 is a receptor for neutrophils and monocytes when it is expressed on activated platelets and endothelium. This property facilitates rapid adhesion of leukocytes to endothelium at regions of tissue injury as well as platelet-leukocyte interactions at sites of inflammation and hemorrhage. Like other leukocyte adhesion molecules, GMP-140 may also participate in pathologic inflammation, thrombosis, and tumor metastasis. Confirmation of such pathologic roles may lead to design of new drugs that block adhesive receptor function in human disease.

Key words: selectins, inflammation, hemostasis, leukocytes, cell-cell interactions

Endothelial cells, platelets, and leukocytes are all key participants in hemostatic and inflammatory responses to tissue injury. Many of these responses require cellular activation as well as adhesive interactions among cells. This review will focus on the properties of GMP-140, a receptor that mediates binding of leukocytes to activated platelets and endothelium. These properties will be reviewed in the context of those of other molecules with related structure and/or function.

TISSUE AND SUBCELLULAR DISTRIBUTION OF GMP-140

GMP-140 was originally identified by monoclonal antibodies that reacted only with platelets activated with agonists such as thrombin [1]. Immunogold studies indicated that the protein was localized to membranes of α granules in unstimulated platelets but was rapidly redistributed to the cell surface following activation-induced fusion of granule membranes with the plasma membrane [2,3]. Because the protein has an apparent Mr of 140,000, it was named GMP-140 to indicate that it is a granule membrane protein of 140 kd [2]. The molecule has also been referred to as PADGEM protein [3] and has been given the cluster designation CD62.

Immunoperoxidase analysis of normal human tissues revealed that GMP-140 is present not only in platelets and their precursor cells, megakaryocytes, but also in endothelial cells. Notably, the endothelial protein is primarily found in postcapillary venules, rather than in larger veins, arterioles, or arteries [4]. The protein is also synthesized by human umbilical vein endothelial cells, both in vivo and in vitro, which has facilitated studies of its properties [4]. Endothelial GMP-140 is located in membranes of Weibel-Palade bodies, the secretory granules of endothelium in which large multimers of von Willebrand factor are stored [4–6]. Following cellular stimulation with agonists such as thrombin or histamine, GMP-140 is rapidly redistributed to the cell surface [4,5]. In cultured endothelium, the surface appearance is transient, peaking be-

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tween 3 and 10 min, then declining to basal levels over the next 30 min as a result of endocytosis [5]. When platelets are activated in vitro, GMP-140 remains on the surface for at least 1 h [7]; it is not known if endocytosis occurs more readily in stimulated platelets that remain in the circulation.

Thus, GMP-140 is localized in secretory granule membranes of two cell types in the vascular system, platelets and endothelium (Fig. 1). Following stimulation of these cells with agonists such as thrombin, it is rapidly translocated to the cell surface. These properties make monoclonal antibodies to GMP-140 useful probes of cellular activation in vitro [7] and potential markers of thrombi in vivo [8]. Its rapid inducible expression also suggested that GMP-140 could be an important receptor at sites of tissue injury, where platelets and endothelial cells are activated.

STRUCTURE OF GMP-140

GMP-140 is synthesized by cultured endothelial cells and by HEL cells, a human cell line with features of megakaryocytes [4,9]. Three to four protein precursors of slightly different apparent Mr are immunoprecipitated from metabolically labeled HEL cells. Core high-mannose N-linked oligosaccharides are initially added to the protein core and then modified into larger complex chains. The mature molecule contains 30% carbohydrate by weight [9].

The cDNA-derived amino acid sequence [10] predicts that GMP-140 is an asymmetric molecule composed of several cysteine-rich, independently folded domains (Figs. 2, 3). Beginning at the N-terminus, there is a cleavable signal peptide, a domain homologous to Ca^{2+}-dependent lectins such as the asialoglycoprotein receptor, an epidermal growth factor (EGF)-like module, nine tandem consensus repeats related to those of complement-binding proteins such as CR1, a transmembrane region, and a short cytoplasmic tail. Two variant cDNAs were also identified, one rare form with a deletion encoding the seventh consensus repeat and a more common variant with a deletion encoding the transmembrane domain (Fig. 2). The latter predicts a soluble form of the molecule. These variants may explain the heterogeneous precursors synthesized by HEL cells. The predicted soluble form of GMP-140 is of particular interest, but it remains to be characterized at the protein level.

The human gene for GMP-140 has been mapped to the long arm of chromosome 1 at bands q21-24 [11]. The gene spans over 50 kb and contains 17 exons [12]. As is shown in Figure 3, individual exons tend to encode structurally distinct domains, supporting the concept that GMP-140 evolved as a result of exon duplication and shuffling. The regions deleted in the variant cDNAs are precisely encoded by exons, suggesting that the variants arise by alternative splicing of precursor RNA.

GMP-140 shares extensive sequence similarity and domain organization with two other vascular cell surface molecules, ELAM-1 [13] and the peripheral lymph node homing receptor (murine Mel 14 antigen, human Leu 8 antigen, or LAM-1) [14–16]. These molecules define a

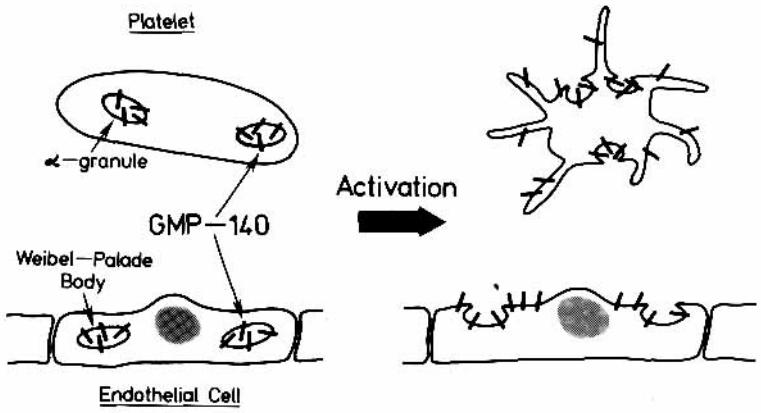

Fig. 1. Redistribution of GMP-140 following cellular activation. In unstimulated platelets and endothelial cells, GMP-140 is located in membranes of secretory granules: α granules in platelets and Weibel-Palade bodies in endothelial cells. When these cells are stimulated, the granules rapidly fuse with the plasma membrane, release their contents, and express GMP-140 on the cell surface. (Reproduced from McEver [36] with permission of Springer-Verlag.)

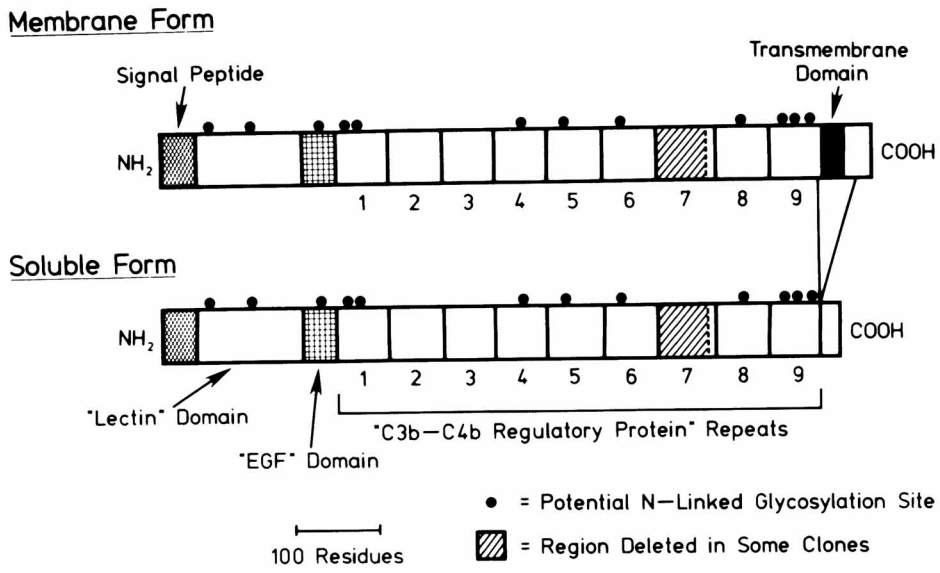

Fig. 2. Schematic diagram of the domains in GMP-140. (Reproduced from Johnston et al. [10] with permission of Cell Press.)

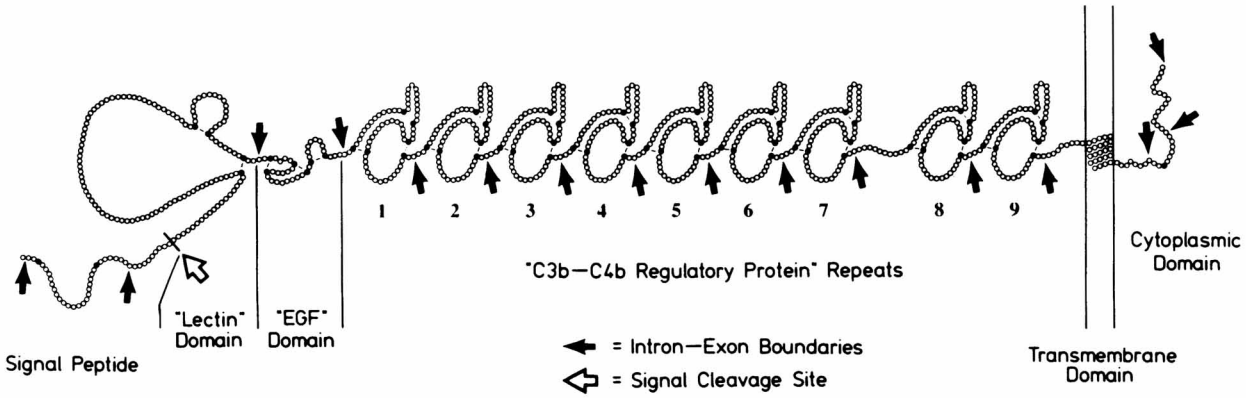

Fig. 3. Exon-intron boundaries of the gene for GMP-140, shown in relation to the structural domains of the encoded protein. The predicted disulfide bond patterns in each domain are also illustrated. (Reproduced from Johnston et al. [12] with permission of the Journal of Biological Chemistry.)

new gene family, termed *selectins,* each of which contains an N-terminal lectin-like domain, followed by an EGF-like domain, consensus repeats (nine in GMP-140, six in ELAM-1, and two in the homing receptor), a transmembrane domain, and a short cytoplasmic tail. The genes have similar exon-intron boundaries, supporting their evolution by exon shuffling [12,17,18]. Furthermore, all three genes are tightly clustered in a 300 kb region in equivalent regions of murine and human chromosome 1 [11]. Interestingly, the genes encoding complement-regulatory proteins, which contain similar consensus repeats, are clustered at a slightly different region of chromosome 1 [11].

GMP-140 IS A RECEPTOR FOR NEUTROPHILS AND MONOCYTES

The selectins share functional as well as structural similarity. All three molecules mediate interactions of leukocytes with the blood vessel wall during immune or inflammatory responses. The term *selectins* was proposed because the molecules mediate selective cell-cell contacts by possible lectin-like mechanisms. The homing receptor, reviewed elsewhere in this issue, mediates lymphocyte adhesion to high endothelial venules of peripheral lymph nodes by a process that appears to include carbohydrate recognition. It is also expressed on neutrophils and

monocytes, where it may support adherence to an unknown receptor on cytokine-activated endothelium [19]. ELAM-1 (endothelial leukocyte adhesion molecule-1) is transiently expressed by cytokine-activated endothelium, where it binds neutrophils and monocytes [13].

Like ELAM-1, GMP-140 is a receptor for neutrophils and monocytes (Fig. 4). Neutrophils bind to purified, immobilized GMP-140 [20], to COS cells transfected with GMP-140 cDNA [20], and to activated platelets or endothelium expressing GMP-140 [20–22]. These interactions are inhibited by monoclonal antibodies to GMP-140 and by fluid-phase GMP-140. Radiolabeled GMP-140 binds to a saturable number of receptors on neutrophils and monocytes but not lymphocytes or platelets. The receptors have not been identified, but it is known that neutrophil activation does not alter their number or the apparent affinity of their interaction with GMP-140 [23]. Furthermore, GMP-140 binds equally effectively to fixed or chilled neutrophils, suggesting that metabolic energy is not required for receptor function [20,23].

The specific regions of GMP-140 that mediate leukocyte recognition have not been identified. The lectin-like domain is a candidate for several reasons. First, its N-terminal location positions it favorably for interaction with another cell. Second, evidence supporting carbohydrate recognition in homing receptor-mediated adhesion [24] suggests that the lectin domains of all the selectins, including GMP-140, participate in cell-cell contact. Third, leukocyte recognition by GMP-140 requires Ca^{2+}, consistent with the Ca^{2+} dependence of known lectins with related structure [20–22]. However, at least two divalent cation-binding sites are involved in GMP-140-dependent adhesion [20]. Ca^{2+} is known to bind to EGF-like domains of other proteins, suggesting that the EGF-like domain of GMP-140 might also participate in leukocyte recognition in a Ca^{2+}-dependent manner. The EGF-like region may bind to a site on the receptor distinct from that identified by the lectin-like domain, or it could modulate the leukocyte-recognition properties of the lectin-like domain. The consensus repeats might simply position the lectin- and EGF-like domains far enough from the membrane for optimal binding to another cell. Alternatively, they may have other functions not yet identified.

PHYSIOLOGIC ROLE OF GMP-140

During inflammation, leukocytes must first adhere to endothelium, then migrate into tissues at sites of infection or injury. In vivo, this is a temporally regulated process, with emigration of neutrophils and monocytes occurring earlier than lymphocytes. In inflammatory sites exposed to mediators such as thrombin or histamine, GMP-140 is rapidly expressed on the endothelial cell surface, making it an excellent candidate for directing adherence of unstimulated neutrophils and monocytes to endothelium within minutes after tissue injury. Expression of GMP-140 is transient, leading to dampening of the proinflammatory surface within 30–60 min. However, persistent infection or injury leads to release of inflammatory cytokines that induce endothelial expression of ELAM-1 over a time course of several hours. GMP-140 and ELAM-1 are preferentially expressed in postcapillary venules, the favored sites of leukocyte extravasation during inflammation [4,25]. Both are receptors for neutro-

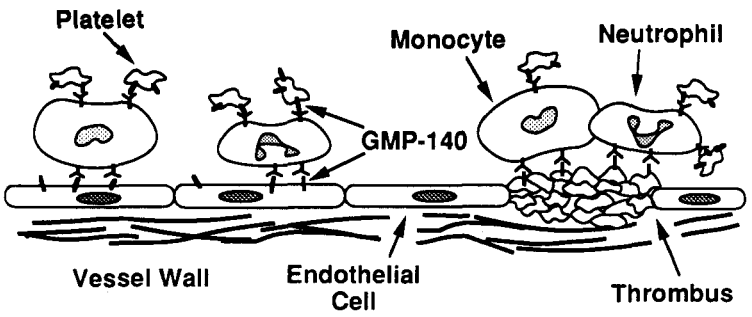

Fig. 4. Adhesion of neutrophils and monocytes to activated platelets and endothelial cells expressing GMP-140. The leukocyte receptors for GMP-140 have not been identified. On the left is an inflammatory site, where leukocytes bind to activated endothelium; the leukocytes, in turn, may recruit activated platelets. Not shown is secondary emigration of leukocytes into tissues beneath the endothelium. On the right is a site of vascular damage and bleeding, where adherent, activated platelets may bind neutrophils and monocytes.

phils and monocytes, but not lymphocytes, consistent with their roles in acute inflammation.

The regulated expression of GMP-140 and ELAM-1 on activated endothelium localizes adhesion of unstimulated neutrophils and monocytes to appropriate inflammatory sites. Emigration into tissues, however, requires transiently induced adhesive competency of the β2 integrins (CD11/CD18 molecules) on activated leukocytes [26]. Thrombin- or histamine-stimulated endothelial cells synthesize a novel lipid mediator, platelet-activating factor (PAF), which is translocated to the cell surface. The expression of PAF, like that of GMP-140, is rapid and transient. Neutrophils bound to GMP-140 on the endothelial cell surface are thus positioned for activation by PAF, leading to mobilization of the β2 integrins and subsequent transendothelial migration [27]. An analogous mechanism may activate neutrophils and monocytes bound to ELAM-1 on endothelium exposed to cytokines.

Chronic inflammation requires the delayed and sustained emigration of mononuclear cells. This process is mediated by cytokine-induced endothelial expression of VCAM-1 (vascular cell-adhesion molecule-1), also known as INCAM-110 (inducible cell-adhesion molecule of 110 kd). VCAM-1, a member of the immunoglobulin superfamily, mediates adhesion by binding to the integrin VLA-4 on lymphocytes and monocytes [28].

Unlike the other selectins, GMP-140 is also found in platelets, where it mediates binding of activated platelets to neutrophils and monocytes [21,22]. This process may be important in both inflammation and hemostasis (Fig. 4). In vivo, neutrophils can support emigration of platelets into acute inflammatory sites [29]; this process might require GMP-140-mediated contacts between neutrophils and activated platelets. The recruitment of platelets may be useful, since these cells release a number of proinflammatory mediators. At sites of hemorrhage, platelets adhere to subendothelial surfaces, undergo activation, and form platelet aggregates. Stimulated platelets expressing GMP-140 may, under suitable flow conditions, recruit neutrophils and monocytes to the thrombus. Activated monocytes develop procoagulant surfaces that amplify thrombin generation and clot formation. Close contact between platelets and neutrophils may lead to transcellular synthesis of novel leukotrienes and other lipid products that are not produced by either cell type alone [30].

Although GMP-140 clearly plays a major role in supporting platelet-leukocyte interactions, other adhesion molecules may participate. One candidate is PECAM-1 (CD31), a member of the immunoglobulin superfamily that is expressed on surfaces of platelets, leukocytes, and endothelium [31]. PECAM-1 appears to mediate homotypic interactions between endothelial cells [32]. It might also stabilize platelet aggregates and platelet-leukocyte contacts. If so, the molecule must require cellular activation before it can support adhesion, since unstimulated platelets and leukocytes, unlike endothelial cells, do not form contacts.

PATHOLOGIC ROLE OF GMP-140

Excessive accumulation of neutrophils, with concomitant release of toxic proteases and oxygen radicals, has been implicated in the pathogenesis of inflammatory disorders such as reperfusion injury, acute respiratory distress syndrome, and rheumatoid arthritis. A number of leukocyte adhesion molecules might be involved in these diseases, including GMP-140 in situations in which activators of platelets and endothelium such as thrombin or histamine might be released. In some pathologic states, nonphysiologic mediators might be generated that can also activate these cells. For example, deposition of the terminal complement proteins C5b-9 on endothelial cells promotes secretion of von Willebrand factor and translocation of GMP-140 [33]. Low concentrations of oxygen radicals induce prolonged exposure of GMP-140 on the endothelial cell surface, leading to enhanced neutrophil adherence over several hours [34]. In vivo, this unregulated expression of GMP-140 could result in sustained neutrophil recruitment, additional production of oxygen radicals by the adherent cells, and eventual tissue destruction.

Some malignant cells may utilize processes normally restricted to leukocyte recruitment in order to promote hematogenous metastases. Lectin-carbohydrate interactions have been proposed to be responsible for spread of experimental malignancies. At least one human colon carcinoma cell line binds specifically to ELAM-1 expressed on activated endothelium [35]. Other malignant cells might express receptors for GMP-140. Adhesion mediated by molecules other than selectins may also occur, as a melanoma

cell line adheres to the Ig-like molecule, INCAM-110 (VCAM-1) [35]. Malignant cell-surface display of receptors for leukocyte adhesion molecules may target spread of tumors to endothelium at inflammatory sites, where expression of the adhesion molecules would be induced. Platelets have also been shown to promote experimental tumor metastasis; there may be examples in which platelet adhesion to tumor cells is mediated by GMP-140.

Appropriate in vivo models of inflammation, metastasis, and thrombosis are required to evaluate the participation of GMP-140 and other adhesion receptors in disease. Should their pathologic roles be confirmed, new pharmaceuticals based on their properties might be designed to interrupt their function. These drugs might include monoclonal antibodies, peptides, oligosaccharides, or recombinant soluble proteins, variously designed to block adhesion molecules on endothelium, leukocytes, or platelets.

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