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REVIEW ARTICLE

Cell Adhesion Molecules in Coronary Artery Disease

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To date, six families of cell adhesion molecules are known. These are cell surface receptors that mediate adhesion of cells to each other or to components of the extracellular matrix and include integrins, selectins, the immunoglobulin superfamily, cadherins, proteoglycans and mucins. These cell adhesion molecules play a key role in cell–cell interaction (such as among endothelium, monocytes, smooth muscle cells and platelets) and cell–extracellular matrix interaction (such as between leukocytes, platelets or fibroblasts and the extracellular matrix). The importance of these interactions has recently been demonstrated in clinical trials with the use of an antibody fragment directed against the platelet αIIbβIIIa integrin, with reduction of arterial thrombosis and restenosis after percutaneous coronary interventions. A fundamental role for cell adhesion molecules has been suggested for several other relevant disease processes, including atherosclerosis, acute coronary syndromes, reperfusion injury and allograft vasculopathy. This review focuses on providing the clinically relevant biology of these families of adhesion molecules, setting the foundation for delineation of their emerging role in cardiovascular therapeutics.

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Adhesion of circulating leukocytes and platelets to the blood vessel wall is an essential component of acute and chronic events in various coronary artery disease processes, including atherosclerosis, thrombosis, restenosis after percutaneous transluminal coronary angioplasty, reperfusion injury and allograft vasculopathy in cardiac transplant recipients. In each step of cell–cell binding and cell–extracellular matrix binding, cell adhesion molecules, including integrins, selectins and immunoglobulin superfamilies, mediate these processes.

Integrins

Integrins were named by Richard Hynes in 1987 (1) for the family of integral membrane receptors thought to "integrate" the cytoskeleton of one cell with that of another cell or with extracellular matrix. Because a number of the proteins were identified before the recognition of an integrin superfamily, some of the subunits and members have multiple names (Table 1). The integrins comprise a superfamily of heterodimer transmembrane proteins composed of noncovalently associated α- and β-subunits (Fig. 1) (2). Presently, eight β-subunits and 12 α-subunits have been identified, and genomic analysis suggests that there may be more integrin subunits (3). Integrins are subdivided into several subfamilies based on the sharing of a common β-subunit by various α-subunits. Among these subfamilies, three main groups are widely known because of their earlier discovery than others. First, *very late appearing antigen* (VLA) integrins have a β_1-subunit and are named because they were first identified on T lymphocytes "very late after" mitogen stimulation (4). Second, *leukocyte integrins* share a β_2-subunit. The β_2-subfamily of leukocyte integrins consists of heterodimers referred to as lymphocyte function-associated antigen (LFA)-1 (also known as $\alpha_L\beta_2$ or CD11a/CD18), Mac-1 ($\alpha_M\beta_2$, CD11b/CD18 or CR3) and p150/95 ($\alpha_X\beta_2$, CD11c/CD18) (5). Third, *cytoadhesins* have a β_3-subunit. The β_3-subfamily of integrins consists of the platelet glycoprotein IIb/IIIa complex and the vitronectin receptor (Table 1) (6).

Many integrins that bind to matrix molecules recognize the tripeptide Arg-Gly-Asp (RGD) (7). These RGD sequences are found within a number of matrix proteins, including fibronectin, fibrinogen, thrombospondin, vitronectin, laminin and type I collagen, although recognition of these matrix proteins by integrins is not always mediated by their RGD sequences. The β_3- and some β_1-integrins recognize RGD sequences as binding sites of these proteins, but the β_2-integrins are not generally thought to recognize the RGD motif (8,9). The largest number of integrins are members of the β_1- or VLA subfamily, which are distributed in various kinds of cells. The VLA integrins mediate cell adhesion to a number of proteins found in extracellular matrix, including collagen, fibronectin and laminin. The β_2- or leukocyte integrin subfamily appears in the leukocytes restrictively (10,11). The ligands identified for the leukocyte integrins are shown in Table 1. These leukocyte integrins show different distributions (12,13): LFA is found on all leukocytes, and Mac-1 and p150/95 are present in myeloid cells and monocytes. Mac-1 is abundant in cells of the myeloid lineage, whereas p150/95 is most highly expressed by tissue

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Table 1. Major Subfamilies of Integrin Molecules and Ligands

Integrin Subfamilies	Ligands	RGD
VLA Integrins (β_1 subfamily. VLAβ, GPIIa, CD29)		
$\alpha_1\beta_1$ (VLA-1, CD49a)	Laminin, collagen	–
$\alpha_2\beta_1$ (VLA-2, GPIa, CD49b)	Collagen, laminin	+
$\alpha_3\beta_1$ (VLA-3, CD49c)	Fibronectin, laminin, collagen	+
$\alpha_4\beta_1$ (VLA-4, CD49d)	Fibronectin, VCAM-1. PP HEV	–
$\alpha_5\beta_1$ (VLA-5, GPIc, CD49e)	Fibronectin	+
$\alpha_6\beta_1$ (VLA-6, GPIc', CD49f)	Laminin	–
Leukocyte Integrins (VLA-5, GPIc, CD49e)		
$\alpha_L\beta_2$ (LFA-1, CD11a)	ICAM-1, ICAM-2	–
$\alpha_M\beta_2$ (Mac-1, CD11b)	ICAM-1, iC3b, fibrinogen, Factor X	–
$\alpha_X\beta_2$ (p150, CD11c)	Fibrinogen, iC3b?	–
Cytoadhesins (β_3-subfamily, cytoadhesins, GPIIIa, CD61)		
$\alpha_{IIb}\beta_3$ (GP IIb, CD41)	Fibrinogen, fibronectin, vitronectin, von Willebrand factor	+
$\alpha_v\beta_3$ (vitronectin receptor, CD51)	Vitronectin, fibrinogen, von Willebrand factor, thrombospondin, osteopontin	+

CD = cluster determinant; GP = glycoprotein; ICAM = intercellular adhesion molecule; iC3b = breakdown product of the third component of complement; LFA = lymphocyte function-associated antigen; VCAM = vascular cell adhesion molecule; VLA = very late after activation. (Modified from Shimizu Y, Shaw S. Lymphocyte adhesion mediated by VLA [beta 1] integrins. In: Hogg N, editor. Integrins and ICAM-1 in Immune Responses. Chemical Immunology, Vol. 50. Basel: Karger, 1991:34–54. Reprinted with permission.)

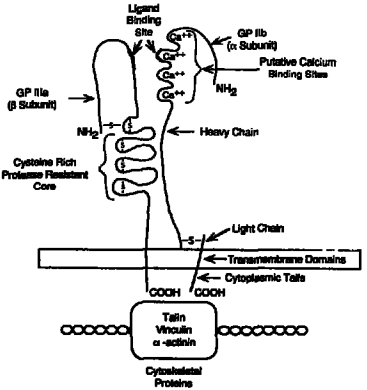

Figure 1. Schematic structure of a prototype integrin, platelet glycoprotein IIb/IIIa. Integrins are composed of two noncovalently bound subunits designated as α and β. The β-subunit consists of an extracellular amino terminus, a disulfide loop that links to its midregion, three or four disulfide-rich protease-resistant domains, a transmembrane region and a short cytoplasmic tail. The α-subunit consists of disulfide-linked heavy and light chains; the heavy chain contains four divalent cation-binding portions, and the light chain contains the transmembrane segment and a short cytoplasmic tail. The cytoplasmic tails of the integrin are bound to cytoskeletal elements, such as talin, vinculin and α-actinin, that link the integrin with the actin cytoskeleton. (Modified from Plow EF, D'Souza SE, Ginsberg MH. Ligand binding to GPIIb-IIIa: a status report. Semin Thromb Hemostas 1992;18:324–32. Reprinted with permission.)

macrophages. GPIIb/IIIa ($\alpha_{IIb}\beta_3$, CD41/CD61) is primarily found on megakaryocytes and platelets. Recently, GPIIb/IIIa has also been shown to be expressed by certain tumor cells (14). The activation of GPIIb/IIIa by various agonists including adenosine diphosphate (ADP), epinephrine, thrombin and collagen is the final common pathway for platelet aggregation. This activation event also permits GPIIb/IIIa to bind fibronectin and von Willebrand factor (vWF), mediating the adhesion of platelets to endothelium or the subendothelial matrix (15). The vitronectin receptor (VNR, $\alpha_v\beta_3$ or CD51/CD61) has the widest distribution, appearing on most mesenchymal cells as well as on many other kinds of cells (7). The vitronectin receptor also binds to multiple ligands, including vitronectin, fibrinogen, thrombospondin, von Willebrand factor and osteopontin.

Selectins

The selectins have been called by various names according to their discovery (16). L-selectin has been termed the lymphocyte-homing receptor, $gp90^{MEL}$, Leu-8, leukocyte adhesion molecule (LAM)-1, leukocyte endothelial cell adhesion molecule (LECAM)-1 and MEL-14. E-selectin was called endothelial leukocyte adhesion molecule (ELAM)-1. P-selectin was called CD62, granule membrane protein of molecular mass 140 kilodaltons (GMP 140) and platelet activation-dependent granule external membrane protein (PADGEM). A standard nomenclature has been agreed on (17), which designates each family member according to the cell type on which it was originally identified: E-selectin (endothelium), P-selectin (platelets) and L-selectin (lymphocytes) (Table 2).

The selectins are composed of a lectin domain, an epithelial growth factor (EGF)-like region and complement regulatory-like modules (16,18) (Fig. 2). P- and E-selectins bind to common sites of carbohydrates, including sialylated Lewis x (sLe^x)-related structures, sulfated polysaccharides (heparin, fucoidan) and phosphated monosaccharides and polysaccharides (16,19,20). L-selectin binds to mucin-like endothelial glycoproteins (21). E-selectin has been known to participate in the adhesion of neutrophils, monocytes and a subpopulation of memory T lymphocytes to endothelial cells that have been activated by cytokines (interleukin-1, tumor necrosis factor) and bacterial endotoxin (22). Eosinophils (23) and basophils (24) may also bind to endothelium through E-selectin. P-selectin (CD62) is present in alpha-granules of platelets at rest

Table 2. Selectins: Nomenclature and Expression

	Cell Distribution	Regulation	Binds to
L-selectin	All leukocytes	Constitutive surface expression; modulation of activity; shed after cellular activation	LNHEV, activated endothelial cells
E-selectin	Activated endothelial cells	IL-1, TNF, LPS; inducible expression (h); RNA; protein synthesis	Neutrophils, monocytes, some T cells
P-selectin	Platelets, Weibel–Palade bodies of endothelial cells	Thrombin, histamine; others from storage granules (min); cytokine inducible (?)	Neutrophils, monocytes, some T cells

IL-1 = interleukin-1; LNHEV = lymph node high endothelial venules; LPS = lipopolysaccharide; RNA = ribonucleic acid; TNF = tumor necrosis factor. (Modified from Bevilacqua MP. Endothelial-leukocyte adhesion molecules. Annu Rev Immunol 1993;11:767–804. Reprinted with permission.)

Table 3. Immunoglobulin Superfamily

Major Glycoprotein	Primary Distribution	Counter-receptor	Time of Maximal Expression
ICAM-1	Endothelial cells, fibroblasts, epithelial cells, hematopoietic cells	LFA-1, Mac-1	12–24 h
ICAM-2	Same as ICAM-1	LFA-1	Constitutive
VCAM-1	Endothelial cells, smooth muscle cells	VLA-4	4–10 h
PECAM-1 (CD31)	Intercellular junction of endothelial cells, platelets		

PECAM = platelet endothelial cell adhesion molecule; other abbreviations as in Table 1. (From Faruqi RM, Dicorleto PE. Mechanisms of monocyte recruitment and accumulation. Br Heart J 1993;69 Suppl:S19–29. Reprinted with permission.)

(25) and Weibel–Palade bodies (26) of endothelial cells. On activation by thrombin or other mediators, P-selectin is rapidly redistributed to the surfaces of platelets and endothelial cells (27,28). P-selectin has been shown to bind to different types of leukocytes, including neutrophils and monocytes (27,29,30), thus mediating platelet–leukocyte and endothelial cell–leukocyte interactions. L-selectin, found on most circulating human lymphocytes, neutrophils and monocytes, was initially implicated in lymphocyte homing to secondary lymphoid tissues (31,32). Recent studies (33,34) in vivo have suggested that L-selectin has also participated in leukocyte rolling on the vessel wall as well as the extravasation process during inflammation. Unlike the other two selectins, L-selectin is constitutively expressed and shed from the cell surface within minutes after leukocyte activation (35).

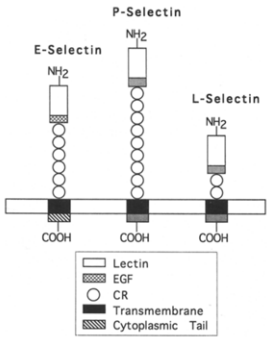

Figure 2. Schematic presentation of E-, P- and L-selectins showing an amino terminal lectin domain, epidermal growth factor (EGF)-like region, discrete number of complement binding-like proteins, transmembrane region and a cytoplasmic tail. CR = complement regulatory-like modules. (Modified from Bevilacqua MP. Endothelial-leukocyte adhesion molecules. Annu Rev Immunol 1993;11:767–804. Reprinted with permission.)

Immunoglobulin Superfamily

In the 1980s, it became apparent that certain members of the immunoglobulin superfamily, including intercellular adhesion molecule (ICAM)-1, ICAM-2 and vascular cell adhesion molecule (VCAM)-1, play key roles not only in adhesion but also in transmigration of blood leukocytes (36) (Table 3). ICAM-1 (CD54) and ICAM-2 are closely related in structure and function (Fig. 3). ICAM-1 is widely expressed by hematopoietic and nonhematopoietic cells and is a primary ligand for LFA-1 (37). ICAM-2 is also a ligand for LFA-1, and its distribution in T, B and monoblastic cell lines is suggested by the generation of its messenger ribonucleic acid (38).

Regulation of ICAM-1 expression can be achieved by treatment with specific cytokines (tumor necrosis factor [TNF]-α, interferon-γ and interleukin [IL]-1) or by adherence to matrix proteins such as fibronectin (39,40). Like E-selectin, ICAM-1 is expressed in abundance on vascular endothelium several hours after stimulation by interleukin-1 or tumor necrosis factor (39,40). However, the pattern of expression of ICAM-1 shows differences from that of E-selectin (Tables 2 and 3).

Recently, it was shown that a ligand for $\alpha_4\beta_1$ (VLA-4: CD49d/CD29)-integrin is one of the immunoglobulin superfamily members, VCAM-1 (41). Endothelial VCAM-1 was first shown to support the adhesion of lymphocytes and monocytes through an interaction with the integrin $\alpha_4\beta_1$ (42,43). In addition, adhesion of eosinophils (44) and basophils, but not neutrophils (45), to activated endothelium also appears to

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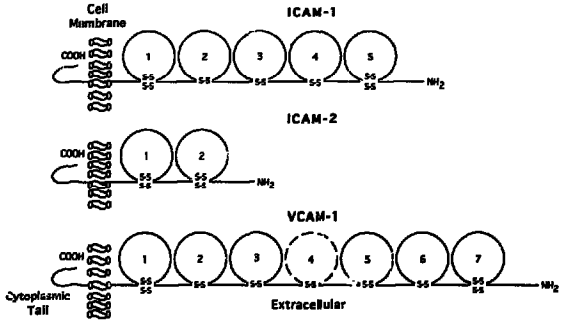


Figure 3. Structure of intercellular adhesion molecule (ICAM)-1, ICAM-2 and vascular cell adhesion molecule (VCAM)-1. ICAM-1 has five immunoglobulin-like domains (**disulfide-linked circle**) followed by a transmembrane region and a short cytoplasmic tail. ICAM-2 has only two extracellular immunoglobulin domains, and VCAM-1 has either six or seven immunoglobulin domains. **Dashed circle** shows the one domain that is variably present. (Modified from Hogg et al. [36]. Reprinted with permission.)

invoive VCAM-1. VCAM-1 is also expressed on several nonvascular cell types (46). On stimulation by IL-1 and TNF-α, VCAM-1 on the endothelial cell surfaces is up-regulated, and maximal activity is reached by 6 to 12 h (47).

Platelet endothelial cell adhesion molecule (PECAM)-1 (CD31) has been shown to mediate endothelial cell–cell interactions (48) as a homotypic cell adhesion molecule. In addition, its presence on platelets, T cells and monocytes suggests that it may have an important role in endothelialization, thrombosis (49) and transendothelial migration of leukocytes (50).

Other Cellular Adhesion Molecules

The cadherin superfamily is a group of proteins for maintaining tight gap junctions and intercellular spacing in adult tissue (51,52). Proteoglycans constitute a large protein family with glycosaminoglycan side chains mediating lymphocyte binding to mucosal high endothelial venules (53) and epithelial cells binding to collagens I, III and V, fibronectin and thrombospondin (54). Platelet GPIb-IX complex, the most familiar cell adhesion molecule of the mucin family, contains a thrombin-binding site and a von Willebrand factor-binding site (55), which is responsible for initial binding of the platelet at rest to subendothelium (56).

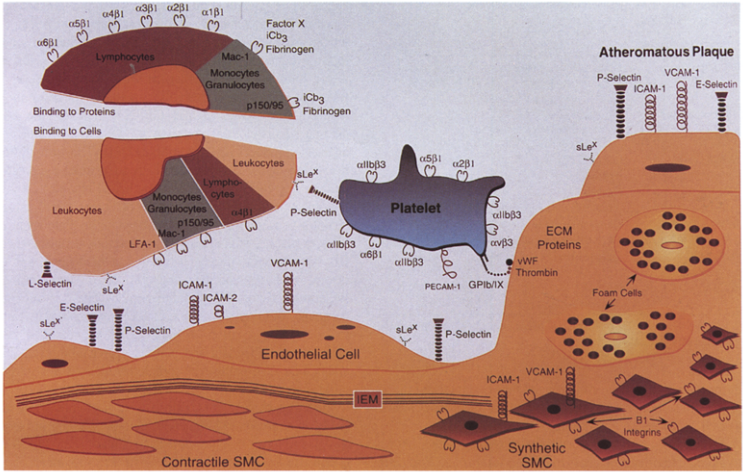
Role in Coronary Artery Disease

Atherosclerosis

The basic pathogenesis of the atherosclerotic process has been described in detail by Ross (57) and updated recently (58). The fundamental atherosclerotic lesion is the fibrofatty plaque, which predisposes to thrombosis and calcification and ultimately results in narrowing of the arteries. The cell components in the atherosclerotic lesion consist principally of smooth muscle cells, macrophages and some T lymphocytes (59). The roles of a number of cell adhesion molecules in this complex interaction are critical (Fig. 4). The adhesion proteins expressed on the vascular endothelial cell surface fall into two classes: the selectins (E-selectin, P-selectin) and the immunoglobulin family (VCAM-1, ICAM-1). Recently, the role of cell adhesion molecules in leukocyte–endothelial cell interactions and regulation of leukocyte migration was precisely reviewed by Adams and Shaw (60). On leukocytes the LFA-1, Mac-1 and p150/95 integrins and L-selectin have been implicated. In human atheroma, VCAM-1 is expressed not only on endothelial cells but also on macrophages and smooth muscles (61). Clyman et al. (62) demonstrated that smooth muscle cells of the blood vessel express various β_1-integrins that appear to mediate cell interactions with the extracellular matrix. Boudreau et al. (63) showed that addition of RGD peptides or antibody against fibronectin decreased migration of smooth muscle cells.

The pathogenic role of lipids has been studied extensively in atherosclerosis. The lipids participate in a cycle of local cytokine elaboration. The cytokines, such as IL-1 and TNF-α, activate monocytes and macrophages (64). Macrophages can modify low density lipoprotein (LDL) by peroxidation (65). It is suggested that oxidized LDL has cytotoxic effects on endothelial and mesenchymal cells and is responsible for the central necrosis that frequently exists in advanced atherosclerotic plaques. Oxidized LDL also amplifies the release of local cytokines elaborated by endothelial cells. IL-1 and TNF-α cause a rapid induction of the adhesion molecules E-selectin, VCAM-1 and ICAM-1, whereas interferon-γ up-regulates ICAM-1 over a longer period, ~10 to 24 h (40,66).

Li et al. (67) showed that atherogenic diets can induce VCAM-1 expression on vascular endothelial cells as well as smooth muscle cells in intima. They also showed a time gap between expression of VCAM-1 between endothelial cells, where it appears within the first week, and smooth muscle cells, which do not appear until after 6 weeks of atherogenic diet. Expression of VCAM-1 and the presence of T cells in athero-



sclerotic plaque suggest that immunologic events mediated by cell adhesion molecules may play a role in the progression of atherosclerosis. In addition, on the basis of the time gap of VCAM-1 expression between endothelium and smooth muscle cell, VCAM-1 may be a marker for "activation" of smooth muscle cells.

As a result of increased cell adhesion molecules on the activated endothelium, binding of leukocytes and monocytes is potentiated. Some cytokines or even the modified lipids may act as monocyte chemoattractants, which allow monocytes to enter the intima by squeezing between endothelial cells. Schwartz et al. (68) showed that monocytic migration into the subendothelial space through cell junctions occurred in response to chemoattractants that may be present in the intima or media of the vessel wall. Using an antibody probe, Muller et al. (69) showed the importance of PECAM-1 in transendothelial migration of leukocytes. The macrophages in the subendothelial layer have been shown to produce several growth factors, such as platelet-derived growth factor (PDGF) (70) and transforming growth factor (TGF) (71). Monocyte-derived TGF-β stimulates matrix production by smooth muscle cells (72). Then smooth muscle cells are surrounded by the extracellular matrix, which is composed of fibronectin, laminin and collagen types I and IV (73). These extracellular matrix proteins not only promote cell adhesion and migration (74) but also influence the phenotypic transformation of the smooth muscle cell (75) by means of the β_1-integrin subfamily; fibronectin, which is increased in artherosclerosis, promotes modulation of the smooth muscle cells from a contractile to synthetic phenotype, whereas laminin has the opposite effect (76). The β_1-integrins permit smooth muscle cells to attach to

Figure 4. Schematic illustration of cell adhesion molecules on leukocytes, endothelial cells, platelets and smooth muscle cells (SMCs). In leukocytes, several β_2-integrins, which bind to various extracellular matrix proteins, are expressed in T and B lymphocytes. The $\alpha_4\beta_1$-integrin, which binds fibronectin, also mediates direct interaction between lymphocytes and tissue cells through its binding of vascular cell adhesion molecule (VCAM)-1. The lymphocyte function-associated antigen (LFA)-1 integrin, present on most leukocytes, binds to intercellular adhesion molecule (ICAM)-1 and ICAM-2 on the endothelium. Mac-1, the most abundant integrin on neutrophils, interacts with ICAM-1, iC3b, fibrinogen and factor X. p150/95, present on granulocytes and monocytes, also binds to iC3b and fibrinogen. Mac-1 and p150/95 may also bind to other cell surface ligands that are important in neutrophil and monocyte adhesion and extravasation. L-selectin, constitutively expressed on leukocytes, binds to sialylated Lewis structures (sLe^x) and participates in leukocyte rolling. E-selectin, which is essential for leukocyte rolling, and P-selectin, which mediates leukocyte–endothelial interaction, are expressed on endothelial cells. On platelets, $\alpha_{IIb}\beta_3$ is the most abundant integrin and mediates platelet adhesion and aggregation. $\alpha_v\beta_3$ and sparse expression of several β_1-integrins mediate platelet adhesion to extracellular matrix proteins, and the glycoprotein Ib/IX binds to von Willebrand factor (vWF) and thrombin. During the atherosclerotic process, ICAM-1, VCAM-1, E-selectin and P-selectin are up-regulated by cytokines, and quiescent contractile smooth muscle cells are transformed to synthetic proliferative smooth muscle cells by cytokine and growth factors. β_1-integrins on the smooth muscle cell surfaces are implicated in smooth muscle cell–extracellular matrix protein interactions and smooth muscle cell migration. IEM = internal elastic membrane.

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Table 4. Platelet Membrane Glycoproteins and Their Receptor Function

Glycoprotein Receptor	Ligand	Platelet Function
GPIIb:IIIa ($\alpha_{IIb}\beta_3$)	Fibrinogen, von Willebrand factor, fibronectin, thrombospondin, vitronectin	Aggregation, adhesion
Vitronectin receptor ($\alpha_v\beta_3$)	Vitronectin, von Willebrand factor, fibronectin, fibrinogen, thrombospondin	Adhesion
GPIa/IIa ($\alpha_2\beta_1$)	Collagen	Adhesion
GPIc/IIa ($\alpha_5\beta_1$)	Fibronectin	Adhesion
GPIc'/IIa ($\alpha_6\beta_1$)	Laminin	Adhesion
GPIb/IX	von Willebrand factor, thrombin	Adhesion
GPV	Thrombin substrate	?
GPIV (GPIIb)	Thrombospondin, collagen	Adhesion
P-selectin	Sialylated Lewis x	Platelet-leukocyte interaction
PECAM (CD31)	PECAM	Platelet-platelet binding

Abbreviations as in Tables 1 and 3. (Modified from Kieffer and Phillips [56]. Reprinted with permission.)

fibronectin, laminin and collagen and may be important in the process of smooth muscle cell migration in the atherosclerotic as well as restenotic process (62).

Certain viral infections of endothelial cells have been shown to increase their adhesiveness to monocytes through P-selectin expression (77). These in vitro findings support the possibility of a role for viral infection in the pathogenesis of atherosclerosis, as has been proposed by several investigators (78,79).

With the lipid hypothesis of atherosclerosis, the thrombogenic or incrustation theory (80,81) suggests the importance of thrombosis in the development of atherosclerotic lesions, as well as platelets as a source of several smooth muscle cell mitogens. The roles of cell adhesion molecules for intercellular adhesion between platelet and endothelium and platelet and leukocyte are discussed later.

Thrombosis

The role of thrombus in the atherosclerotic and restenotic process after coronary angioplasty is significant and is central in acute myocardial infarction. Cell adhesion molecules are involved in thrombus formation through the coagulation pathway with leukocyte integrins and through platelet adhesion and aggregation with β_1- and β_3-integrin.

Platelet and cell adhesion molecules. Platelets contribute to normal hemostasis by adhering to subendothelial surfaces after blood vessels have been damaged and then aggregating to form a thrombus (15). Platelets possess a number of cell adhesion molecules, such as β_1- and β_3-integrins, P-selectin (CD62), PECAM (CD31, a member of immunoglobulin family) and CD36 (glycoprotein IV, a receptor for collagen and for thrombospondin) (56) (Table 4).

The many receptors involved in platelet adhesion, such as GPIa/IIa ($\alpha_2\beta_1$), GPIc/IIa ($\alpha_5\beta_1$, fibronectin receptor), GPIc'/IIa ($\alpha_6\beta_1$, laminin receptor) and $\alpha_v\beta_3$ (vitronectin receptor), appear to be functional on platelets at rest and thus are ready to mediate platelet binding to extracellular matrix (Fig. 4). Under normal circumstances, the intact endothelium sequesters the adhesive glycoprotein ligands (e.g., von Willebrand factor, fibronectin and collagen) from the platelet in the subendothelium, thus preventing platelet adhesion in the absence of vascular damage. GPIb/IX mediates the initial binding of platelets at rest to von Willebrand factor. This interaction induces the transition of GPIIb/IIIa from an inactive to an active state, which leads to platelet aggregation. Platelet aggregation is mediated by ligand binding to GPIIb/IIIa. In contrast to the other platelet receptors, this β_3 integrin receptor is present in an unactivated state on platelets at rest (2). On platelet stimulation with numerous agonists, GPIIb/IIIa becomes activated such that it can bind fibrinogen and several other ligands, including fibronectin, vitronectin, von Willebrand factor and thrombospondin. The activation and occupancy of GPIIb/IIIa is the final common pathway leading to platelet aggregation. A number of agonists can cause exposure of GPIIb/IIIa and platelet aggregation, even if the arachidonic acid pathway is completely blocked by aspirin or other inhibitors.

Recently, blockade of GPIIb/IIIa has been intensively studied as an approach to antiplatelet therapy. The murine monoclonal antibody 7E3, developed by Coller (82), blocks the GPIIb/IIIa receptor. The $F(ab')_2$ or Fab fragment of this antibody can completely block platelet aggregation induced by all agents both ex vivo and in vivo (83). In a pilot study with 7E3, Gold et al. (84) showed an encouraging reduction of the need for coronary revascularization in patients with refractory unstable angina compared with those receiving placebo. Kleiman et al. (85) performed the pilot study of the Thrombolysis and Angioplasty in Myocardial Infarction (TAMI)-8 trial also using 7E3 and showed improved vessel patency and a tendency toward less frequent recurrent ischemia 24 h after thrombolysis. The antibody approach has also been shown in experimental models to shorten the time to achieve thrombolysis and prevent reocclusion (83,86).

The Evaluation of 7E3 in the Prevention of Ischemic Complication (EPIC) (87) trial, a double-blind, placebo-controlled trial of 2,099 patients undergoing high risk coronary angioplasty treated with c7E3 (chimeric Fab), demonstrated a 35% reduction of major ischemic events (death, myocardial infarction, urgent revascularization) at 1 month for the group receiving a bolus and subsequent 12-h low dose infusion of c7E3 compared with those receiving placebo (Fig. 5, top). This is the first demonstration in patients of a therapeutic use for a specific inhibitor of a cell adhesion molecule.

A number of peptides and nonpeptide mimetic agents that inhibit the GPIIb/IIIa receptor are being evaluated in clinical trials. Examples include Integrelin (COR Therapeutics), a KGD cyclic heptapeptide and the small-molecule nonpeptide mimetic agents, including the RGD MK-383 (Merck, Sharpe & Dohme) and RO-44 (Hoffman-LaRoche, Basel, Switzerland). Rapid clearance and reversibility of these drugs, in contrast to the antibody, are possible benefits that might allow for fine

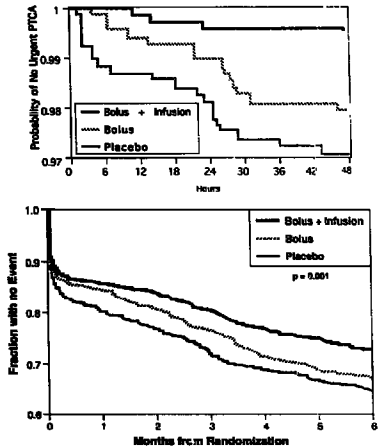

Figure 5. Top, Kaplan-Meier plot demonstrating freedom from urgent repeat percutaneous revascularization procedures from the time of randomization in the EPIC trial. Events began to occur shortly after the index procedure in the placebo group and between 6 and 12 h after the index procedure in the bolus group, whereas events occurred at a later time in the bolus plus infusion group. PTCA = percutaneous transluminal coronary angioplasty. (Adapted from The EPIC Investigators [87]. Reprinted with permission.) **Bottom,** Kaplan-Meier event curve of all events (death, myocardial infarction, coronary revascularization) for patients enrolled in the EPIC trial. There was a significant reduction of events for the c7E3 bolus and infusion group compared with the bolus-only or placebo treatments ($p = 0.001$). A substantial proportion of events occurred after 1 month. (Adapted from Topol et al. [99]. Reprinted with permission.)

titration and the improved control of bleeding complications. By contrast, different members of this class of agents exhibit different binding characteristics and relative specificities. For example, c7E3 also binds to the vitronectin receptor, which may be an important integrin in this setting, but Integrelin does not. The issue of integrin specificity will need to be clarified in future studies.

The coagulation system and cell adhesion molecules. Recently, there have been a few reports of the role of leukocyte integrins in thrombus formation. Both CD11b/CD18 and CD11c/CD18 are fibrinogen receptors expressed by leukocytes. Binding of CD11b/CD18 with immobilized fibrinogen induces the transcription of the tissue factor gene in monocytes, resulting in surface membrane expression of tissue factor and, consequently, the procoagulant activity of these cells (88). In addition to tissue factor, monocytes possess another pathway to initiate coagulation and generate thrombin. Altieri and Edington (89) showed that monocytes can directly activate factor X to Xa after binding this zymogen to a CD11b/CD18. Thrombin generated by the coagulation cascade induces aggregation of platelets to initiate thrombus formation and release inflammatory mediators from platelet granules (90). Thrombin also is a potent chemoattractant for monocytes and smooth muscle cells (91).

Restenosis After Coronary Angioplasty

Restenosis is believed to result from a complex interaction of thrombosis, cellular proliferation and elastic recoil (92). Three phases for developing intimal hyperplasia following vessel injury based on the experimental models (57,93) are postulated. The first phase is characterized by an inflammatory response involving the recruitment of leukocytes to the site of injury and by the formation of thrombus in the first 48 h. A wound matrix, which contains platelets, fibrinogen, fibrin and fibronectin, causes an inflammatory reaction and induces the extravasation of leukocytes, cytokine release, secretion of various growth factors and further generation of thrombin at the site of injury. Through these reactions, endothelium is activated with the expression of adhesion molecules ICAM-1, E-selectin, P-selectin and VCAM-1, and macrophages and fibroblasts begin to migrate into the injury site by means of up-regulation of integrins (94). Di Corleto and de la Notte (95) also showed that thrombin stimulated monocyte adhesion to endothelial cells, probably by triggering expression of P-selectin on the endothelial surface (96). Thus, thrombin generation occurs through the coagulation cascade, and there is recruitment of additional monocytes and platelets and promotion of further platelet aggregation. Platelet binding to the exposed matrix potentially involves the β_1- and β_3-integrins as well as GPIb (6).

The second phase is characterized by the proliferation of smooth muscle cells in the vessel media and the migration of these cells into the intima, where they may proliferate. Platelet aggregation and generation of thrombin at the site of arterial disruption may be important in initiating cellular growth, especially of smooth muscle cells (97,98). The growth factors and cytokines that promote the proliferation and migration of smooth muscle cells are released from the platelet, the leukocyte and even from the smooth muscle cells. Multiple β_1-integrins, which can affect the stability of smooth muscle cell binding to extracellular matrix, are thought to be involved in the process of smooth muscle cell migration (62).

The last phase is the secretory phase of extracellular matrix from smooth muscle cells comprising the neointima. Although the relation between extracellular matrix and cell adhesion molecules in this phase is not well known at present, further studies may disclose the role of cell adhesion molecules in the secretory regulation of smooth muscle cells.

During the 6-month clinical follow-up in the EPIC trial (99), there was a significant 23% reduction in the incidence of ischemic events. The favorable long-term effect was mainly a result of less need for revascularization in patients with an

initially successful procedure (Fig. 5, bottom). The reduction in long-term events after a bolus and 12-h infusion in the acute phase suggests that the phenomenon of arterial "passivation" has taken place, a process by which the arterial surface is transformed from one that supports platelet deposition to one that does not. This is the first large-scale trial to show a significant decrease in clinical restenosis with a pharmacologic intervention, reflected by a decrease in target vessel revascularization. This finding suggests that platelet GPIIb/IIIa integrin blockade may be a useful adjunct to reduce the thrombotic response component of vascular injury. However, it remains likely that an antiproliferative approach, directed at smooth muscle cells, will also be necessary. Large-scale trials of 3,000 to 4,500 patients are presently under way with c7E3 and Integrelin for reduction of clinical and angiographic restenosis.

Reperfusion Injury

Reperfusion injury involves calcium overloading into cells after reoxygenation of ischemic myocardium. Besides the action of calcium, the role of leukocytes in reperfusion injury has been extensively studied, including the contribution of these cells to free radical generation and capillary plugging. A number of studies have shown that depletion or suppression of leukocyte function can reduce the reperfusion injury, including antipolymorphonuclear leukocyte antibodies (100), extracorporeal filtration to remove polymorphonuclear leukocytes (101) and interference with neutrophil functions using various anti-inflammatory agents (102). In a study by Youker et al. (103), it was established that the induction of leukocyte adherence to the cardiac myocyte was promoted during reperfusion. Although the mechanism of cell death in reperfusion injury is still not clear, the role of cell adhesion molecules is implicated in leukocyte adhesion and infiltration as well as phagocytosis. Adhesion molecules known to participate in these reactions are grouped in three broad families.

Yamazaki et al. (104) showed in the rat coronary artery ligation models that pretreatment with monoclonal antibodies against $CD11_a$, $CD11_b$+ $CD11_c$, CD18 and ICAM-1 performed 5 min before coronary occlusion reduces the myocardial infarction size (8.1 ± 1.7%, 10.1 ± 2.1%, 19.6 ± 3.6%, 13.8 ± 2.7%, respectively, compared with the control group, 34.3 ± 4.2%, $p < 0.05$). A study carried out by Ma et al. (105) in a cat reperfusion model also showed that administration of a monoclonal antibody against ICAM-1 10 min before reperfusion leads to significantly less myocardial necrosis than occurs in control animals (10 ± 2% vs. 28 ± 2% of left ventricular area at risk, $p < 0.01$). In contrast to the positive results obtained with leukocyte integrin and ICAM-1 blockade, there is some controversy regarding the role of selectins in reperfusion injury. Weyrich et al. (106) demonstrated that a monoclonal antibody to P-selectin attenuated the polymorphonuclear leukocyte adherence to endothelium and exerted significant endothelial preservation and cardioprotection in myocardial ischemia and reperfusion in cat reperfusion model. However, Kurose et al. (107) showed that a monoclonal antibody against P-selectin or E-selectin did not reduce the leukocyte–endothelial adhesion, although the antibody against L-selectin was effective in a rat reperfusion model. Recently, potential uses of an analog glycoside of the carbohydrate structure sialyl-Lewis X as an antiadhesive agent were suggested in an experimental model (19,20).

Although a number of important issues, such as safety and side effects, have not yet been addressed in anti–cell-adhesion molecules therapy, selected monoclonal antibodies or peptide inhibitors against cell adhesion molecules will be investigated in clinical trials in combination with angioplasty or thrombolysis.

Allograft Vasculopathy in Cardiac Transplantation

Allograft vascular disease, an accelerated form of coronary disease that demonstrates similar pathologic findings to those of early restenosis after angioplasty (108), occurs in heart transplant recipients. Among the various possible mechanisms of this disease, which include the administration of immunosuppressive agents such as corticosteroids, which produce a concomitant hyperlipoproteinemia, viral infections and ischemic injury of coronary artery endothelium occurring between harvest and reimplantation, immune reaction to endothelial cells of the engrafted vessel is accepted as one of the main pathogenetic triggers (109).

The presence of macrophages and T lymphocytes in transplanted coronary arteries (109) and the detection of PDGF at the site of cell proliferation (110) implicate cytokines and growth factors in allograft vasculopathy. The cytokines and the growth factors promote macrophage recruitment and activation, proliferation of smooth muscle cells and extracellular matrix synthesis by smooth muscle cells (64), which are putatively mediated by cell adhesion molecules.

Several studies (111–113) have demonstrated that expression of ICAM-1 and VCAM-1 in capillaries increased with the severity of cellular rejection and was prominent in humoral rejection, suggesting the possibility of monitoring cell adhesion molecules as a means of assessing rejection and the response to therapy. In addition, therapeutic trials in experimental heart transplantation in primates were performed using prophylactic anti–ICAM-1 (114) and anti–VCAM-1 antibody (115); these produced prolonged graft survival without other immunosuppression and suggest that blockade of leukocyte adhesion molecules may hold promise as a therapeutic modality for cardiac allograft vasculopathy.

Conclusions

Cell adhesion molecules are a group of closely related glycoproteins present on a wide variety of cells. The expression and function of these molecules are influenced directly and indirectly by cytokines, growth factors, free radicals and intracellular events, and the role of cell adhesion molecules in the regulation of cellular function must be interpreted in the context of these environmental stimuli. In addition to serving

as a means of recognizing and responding to environmental changes, cell adhesion molecules also control various cell responses such as chemotaxis, migration, differentiation and phagocytosis. Thus, these molecules may play important roles in diverse disease processes, including atherosclerosis, coronary thrombosis, restenosis after percutaneous coronary revascularization, reperfusion injury and cardiac allograft vasculopathy. Investigations in this field are dynamic, and a more comprehensive understanding of the molecules and their functions should promote the development of biologically relevant and potentially more effective therapeutics in the near future.

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