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Review Article

Cell adhesion molecules: The important biomaterials

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ABSTRACT

Cell adhesion molecules (CAMs) are glycoproteins expressed on the cell surface and play a vital role in a wide array of biologic processes. Some of these include: cell growth, differentiation, embryogenesis, immune cell transmigration and response, inflammation, wound healing and cancer metastasis. Adhesion molecules are also capable of transmitting information from the extracellular matrix to the cell. There are four main groups: the integrin family, the immunoglobulin superfamily, selectins, and cadherins. These molecules contribute to the pathogenesis of a large number of common human disorders, such as rheumatoid arthritis, atherosclerosis and tumor cell metastasis in cancer. In this review, the basic mechanisms of cellular adhesion, the characteristics of adhesion molecules, their physiological roles and therapeutic utility are summarized.

Keywords: Adhesion molecules; characteristics; physiological roles; uses

INTRODUCTION

Cellular adhesion is the binding of a cell to a surface, extracellular matrix or another cell using cell adhesion molecules. The adhesion of cells is of crucial importance in governing a range of cell functions in physiology, pathology and biotechnological applications. It is a process that uses not only mechanical interactions but also chemical signals as a basis for cell regulation and has therefore attracted growing attention from all disciplines focused on cell-based biotechnology, including biochemistry, cell biology, biophysics and bioengineering (Orsello et al., 2001). The ability of cells to interact with each other and their surroundings in a coordinated manner depends on multiple adhesive interactions between neighbouring cells and their extracellular environment. These adhesive interactions are mediated by a family of cell surface proteins, termed cell adhesion molecules (Buckley et al., 1998). Thus cell adhesion molecules (CAMs) can be defined as proteins located on the cell surface involved with the binding with other cells or with the extracellular matrix (ECM) in the process called cell adhesion.

Moscona and Moscona (1952a and 1952b) and Townes and Holtfreter (1955) were the scientists who did pioneering work in the identification, characterization and classification of several distinct cell adhesion systems in developing embryos.

CLASSIFICATION

These proteins are typically transmembrane receptors and are composed of three domains: an intracellular domain that interacts with the cytoskeleton, a transmembrane domain, and an extracellular domain that interacts either with other CAMs of the same kind (homophilic binding) or with other CAMs or the extracellular matrix (heterophilic binding).

Adhesion molecules are extremely versatile cell surface receptors which not only stick cells together but provide biochemical and physical signals that regulate a range of diverse functions, such as cell proliferation, gene expression, differentiation, apoptosis and migration.

There are a number of classifications of CAMs. At first they were described on the basis of the tissue in which they were initially identified. It is now clear that they are ubiquitous and not limited to single tissues. For example, E-cadherin, which was initially discovered in the epithelium, has also been localized to non-epithelial tissues; and N-cadherin, which was first discovered in the neural tissues, is also found in epithelium and mesenchyme.

CAMs have also been characterized as calcium-independent (IgSF CAMs and integrins) and calcium-dependent (cadherins and selectins). The distinction is based on whether the presence of calcium is needed for function and for protection from proteolysis. A further classification separates them into primary or secondary groups. Primary CAMs are responsible for specific cell-cell interactions whilst secondary molecules display cell-extra-cellular matrix interactions.

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FAMILIES OF CAMs

More recently, they have been grouped into five superfamilies which contain a number of smaller subfamilies. The superfamilies include the Ig (immunoglobulin) superfamily (IgSF CAMs), the cadherins, the integrins, the selectins and proteoglycans (including the syndecan subfamily of adhesion molecules).

It is suggested that, by controlling the membrane organization of signaling receptors, by imposing spatial organization, and by regulating the local concentration of cytosolic adapter proteins, intercellular and cell-matrix adhesion is more than just glue holding cells together.

IgSF CAMs

A diverse array of cell adhesion receptors are included in the immunoglobulin superfamily of cell adhesion molecules (Ig-CAMs). Proteins of this family are defined by the presence of one or more copies of the Ig fold, a compact structure with two cysteine residues separated by 55 to 75 amino acids arranged as two antiparallel β sheets (Vaughn and Bjorkman, 1996). In many (but not all) cases, CAMs in the Ig superfamily also contain one or more copies of a fibronectin type III repeat domain. Ig family adhesion receptors typically have a large amino terminal extracellular domain, a single transmembrane helical segment, and a cytoplasmic tail. Members of the Ig-CAM family function in a wide variety of cell types and are involved in many different biological processes. Immunoglobulin superfamily CAMs (IgSF CAMs) are either homophilic or heterophilic and bind integrins or different IgSF CAMs.

Some molecules of this family are NCAMs (Neural Cell Adhesion Molecules), ICAM-1 (Intercellular Cell Adhesion Molecule), VCAM-1 (Vascular Cell Adhesion Molecule) and PECAM-1 (Platelet-endothelial Cell Adhesion Molecule).

The immunoglobulins have been studied extensively because of their role in the inflammatory and immune responses. In fact, integrins, selectins, and Ig-CAMs are all critically involved in multiple aspects of immune function (Dustin and Springer, 1991; Rosales C, Juliano, 1995; Springer, 1995). T lymphocytes express several Ig superfamily receptors including CD2, CD4, or CD8, ICAMs 1 and 2, and the T-cell receptor (TCR) itself. These receptors play important roles in antigen recognition, cytotoxic T-cell functions, and lymphocyte recirculation. The members of this polypeptide group differ widely in structure and are able to bind to and neutralize protein toxins, block the attachment of some viruses to cells, opsonize bacteria, activate complement and natural killer cells. The variations possible in the protein binding region of the immunoglobulins allow them to carry out this diverse range of activities. Although they are able to bind to cells, their main role appears to be in the immune and inflammatory responses rather than the maintenance and regulation of the structural components of tissues and organs. However, one of the subfamilies of this group, the carcinoma-embryonic antigen (CEA)-gene family, does appear to have a role in craniofacial development. The cell adhesion molecule C-CAM is a member of this subfamily and can mediate calcium-independent cell-cell adhesion in a homotypic manner. The neural cell adhesion molecule N-CAM is another member of the immunoglobulin superfamily of proteins. There are at least 20 forms of N-CAM, each of which are encoded by several separate genes. N-CAM is expressed in a variety of cell types but has a prominent role in development of the nervous system.

The ICAMs are present on endothelial cells and are recognized by integrins on leukocytes. Other Ig-CAM family adhesion receptors are found on vascular endothelial cells and play an important role in leukocyte trafficking to inflamed tissue sites. For example, vascular cell adhesion molecule-1 (VCAM-1) is an endothelial cell counter-receptor for the integrin α4β1 found on leukocytes. Platelet endothelial cell adhesion molecule-1 (PECAM-1) is an Ig-family cell-cell adhesion molecule that can engage in both homotypic and heterotypic interactions; one of its roles seems to be maintaining tight contacts between adjacent vascular endothelial cells (DeLisser et al., 1994). Ig-CAMs play multiple roles in the developing embryo and in the adult organism. In addition to mediating adhesive contacts that are important in tissue organization, or in cellular trafficking in the immune system, many Ig-CAMs function in key signal transduction processes, as well.

Integrins

The integrins are a family of cell-surface glycoproteins that act as receptors for ECM proteins, or for membrane-bound counter-receptors on other cells. The integrins are a family of heterophilic CAMs that bind IgSF CAMs or the extracellular matrix. Integrin-mediated cell-ECM adhesion sites are complex specialized structures termed focal contacts or focal adhesions (Jockush et al., 1995).

There are many types of integrin, and many cells have multiple types on their surface. Integrins are of vital importance to all animals and have been found in all animals investigated, from sponges to mammals. Integrins have been extensively studied in humans.

Each integrin is a heterodimer that contains an α and a β subunit with each subunit having a large extracellular domain, a single membrane-spanning region, and in most cases (other than β4), a short cytoplasmic domain (Hynes, 1992; Rosales et al., 1992; Ruoslahti, 1991). Twenty-four different alpha subunits that can link in many different combinations with the 9 different beta subunits are known (however not all combinations are observed) which can associate to form more than 20 distinct integrins (Rosales and Juliano, 1995; Hynes, 1992). Both, α and β subunit extracellular domains contribute to the formation of the binding site. The α/β

pairings specify the ligand-binding abilities of the integrin heterodimers. Although the ligands for integrins are often large ECM proteins such as collagen, laminin, vitronectin, or fibronectin, some integrins recognize rather short peptide sequences within the larger protein, for example, the RGD (Arg-Gly-Asp) sequence found in fibronectin and vitronectin. Different Ras-related small GTPases and their downstream effectors can either positively or negatively modulate the ligand binding affinity of integrins (Hynes, 1987).

The ligand binding regions of integrins has three regions which apparently are particularly important: (*a*) a series of seven repeats of approximately 60 amino acids in the N-terminal portion of the a chain, each containing a putative Ca^{2+} binding site; (*b*) an inserted domain (I-domain) of approximately 200 amino acids found in several α chains and containing a nucleotide binding fold and a divalent cation coordination site; (*c*) an I-domain like region of approximately 250 amino acids found in the N-terminal region of the β subunit (Loftus and Liddington, 1997).

The seven-repeat sequences of the α chain usually are involved in protein-protein interactions and form a β-propeller structure with the upper face of the propeller being the ligand binding region and the Ca^{2+} coordination motifs lying on the lower face (Loftus and Liddington, 1997; Springer, 1997). Beta subunits have four cysteine-rich repeated sequences. Both α and β subunits bind several divalent cations. The role of the α-subunit is unknown, but it may stabilize the folds of the protein. The β subunits are more interesting: they are directly involved in coordinating at least some of the ligands that integrins bind.

Integrins work alongside other proteins such as cadherins, cell adhesion molecules and selectins to mediate cell-cell and cell-matrix interaction and communication. They play a role in cell signaling and thereby define cellular shape, mobility, and regulate the cell cycle. Mainly, integrins have two main functions, attachment of the cell to the ECM and signal transduction from the ECM to the cell. However, they are also involved in a wide range of other biological activities, including immune patrolling, cell migration, and binding to cells by certain viruses, such as adenovirus, echovirus, hantavirus, and foot and mouth disease viruses.

A prominent function of the integrins is seen in the molecule GPIIb/IIIa, an integrin on the surface of blood platelets (thrombocytes) responsible for attachment to fibrin within a developing blood clot. This molecule dramatically increases its binding affinity for fibrin/fibrinogen through association of platelets with exposed collagen in the wound site. Upon association with the collagen, GPIIb/IIIa undergoes a conformational change in its structure, allowing it to bind with fibrin and other blood components to form the clot matrix and stop blood loss.

Attachment of cell to the ECM

Integrins couple the ECM outside a cell to the cytoskeleton (in particular the microfilaments) inside the cell. Which ligand in the ECM the integrin can bind to is mainly decided by which α and β subunits the integrin is made of. The connection between the cell and the ECM may help the cell to endure pulling forces without being ripped out of the ECM. The ability of a cell to create this kind of bond is also of vital importance in ontogeny.

Cell attachment to the ECM is a basic requirement to build a multicellular organism. Integrins are not simply hooks, but give the cell critical signals about the nature of its surroundings. Together with signals arising from receptors for soluble growth factors like VEGF, EGF, and many others, they enforce a cellular decision on what biological action to take, be it attachment, movement, death, or differentiation. Thus integrins lie at the heart of many cellular biological processes. The attachment of the cell takes place through formation of cell adhesion complexes, which consist of integrins and many cytoplasmic proteins such as talin, vinculin, paxillin, and alpha-actinin. These act by regulating kinases such as FAK (focal adhesion kinase) and Src kinase family members to phosphorylate substrates thereby recruiting signaling adaptors. These adhesion complexes attach to the actin cytoskeleton. The integrins thus serve to link two networks across the plasma membrane: the extracellular ECM and the intracellular actin filamentous system.

One of the most important functions of surface integrins is their role in cell migration. Cells adhere to a substrate through their integrins. During movement, the cell makes new attachments to the substrate at its front and concurrently releases those at its rear. When released from the substrate, integrin molecules are taken back into the cell by endocytosis; they are transported through the cell to its front by the endocytic cycle where they are added back to the surface. In this way they are cycled for reuse, enabling the cell to make fresh attachments at its leading front.

Signal transduction

Integrins play an important role in cell signaling. Typically, receptors inform a cell of the molecules in its environment and the cell evokes a response. Not only do integrins perform this outside-in signaling, but they also operate an inside-out mode. Thus, they transduce information from the ECM to the cell as well as reveal the status of the cell to the outside, allowing rapid and flexible responses to changes in the environment, for example to allow blood coagulation by platelets.

Connection with ECM molecules can cause a signal to be relayed into the cell through protein kinases that are indirectly and temporarily connected with the intracellular end of the integrin molecule, likely following shape changes directly stimulated by ECM binding.

The signals the cell receives through the integrin can have relation to cell growth, cell division, cell survival, cellular differentiation, apoptosis (programmed cell death) and is involved in processes as diverse as cell migration during embryo-genesis, thrombosis, haemostasis, wound healing, immune and non-immune defence mechanisms and oncogenic transformations.

Cadherins

Cadherins (named for "calcium-dependent adhesion") are a class of type-1 transmembrane proteins. They play important roles in cell adhesion, ensuring that cells within tissues are bound together. They are dependent on calcium (Ca^{2+}) ions to function, hence their name.

The cadherin superfamily includes cadherins, protocadherins, desmogleins, and desmocollins, and more. In structure, they share *cadherin repeats*, which are the extracellular Ca^{2+}-binding domains. There are multiple classes of cadherin molecule, each designated with a prefix (in general, noting the type of tissue with which it is associated). It has been observed that cells containing a specific cadherin subtype tend to cluster together to the exclusion of other types, both in cell culture and during development. For example, cells containing N-cadherin tend to cluster with other N-cadherin expressing cells (homotypic binding). In addition, several groups have observed heterotypic binding affinity (i.e., binding of different types of cadherin together) in various assays (Geiger et al., 1992).

Cadherins can be classified into four groups: classical, desmosomal, protocadherins, and unconventional.

Classical

The structure of a typical classic cadherin consists of an amino-terminal external domain having five tandem repeats, a single transmembrane segment, and a cytoplasmic carboxy-terminal domain of approximately 150 amino acids. The binding functions of the cadherin are localized in the amino-terminal tandem repeat, whereas the other repeats are bridged by calcium binding sites that impart rigidity to the molecule (Aberle et al., 1996). These molecules localize in specialized sites of cell-to-cell adhesion that are termed adherence junctions; at these sites cadherins can establish linkages with the actin-containing cytoskeleton. The cytoplasmic domains of cadherins interact strongly with a group of intracellular proteins known as catenins that are essential for cadherin function (Gumbiner, 1996; Takeichi, 1995). Because there is considerable homology among their cytoplasmic domains, different classic cadherins can compete for the same pool of catenins (Kinter, 1992). The catenins were described initially as a set of three proteins, α-, β-, and γ-catenin (also termed plakoglobin). β-Catenin binds directly to the cadherin cytoplasmic domain; subsequently, α-catenin binds to β-catenin and links the complex to the actin cytoskeleton by direct interaction with actin and by binding α-actinin, an actin-bundling protein (Cowin and Burke, 1996).

Different members of the cadherin family are found in different locations.

- E-cadherin (epithelial): E-cadherins are found in epithelial tissue
- N-cadherin (neural): N-cadherins are found in neurons
- P-cadherin (placental): P-cadherins are found in the placenta.
- R-cadherin (retinal): R-cadherins are found in the retina.
- B-cadherin (brain): B-cadherins are novel chick cadherins found in the brain and epithelial lining of the choroid plexus.

There are many unconventional or ungrouped cadherins like K-cadherin (kidney), H-cadherin (heart), LI-cadherin (liver-intestine), M-cadherin (myotubule) etc.

Classic cadherins play a key role in developmental processes. For example, “knockout” of the gene for E-cadherin (which mediates epithelial interactions) results in an embryonic lethal that arrests before blastocyst formation (Takeichi, 1995).

Desmosomal

Another important subfamily of cadherins involved in adhesion is represented by the desmogleins (DSG1, DSG2, DSG3, DSG4) and desmocollins (DSC1, DSC2, DSC3), a group of desmosome associated cadherins that form intracellular linkages to intermediate filaments rather than actin filaments (Cowin and Burke, 1996).

Protocadherins

Protocadherins were found in vertebrate and invertebrate species. This prevalence in a wide range of species suggested that the fragments were part of an ancient cadherin and were thus termed "Protocadherins" as the "first cadherins". They are the largest subfamily of cadherins present in mammals (Hulpiau and Van Roy, 2009) as differentiators of specific cells. Their function has also been linked to homophilic adhesion, and they have been identified as mediators of this adhesion. Protocadherins can also act as signaling or receptor molecules (Unterseher et al., 2004). Mutations in protocadherin genes and their expression may play a role in schizophrenia (Kalmady and Venkatasubramanian, 2008), Usher Syndrome (Alagramam et al., 2001) and bronchial hyperresponsiveness (Koppelman et al., 2009).

Selectins

Selectins are a family of cell adhesion molecules. The name selectin comes from the words "selected" and

"lectins," which are a type of carbohydrate-recognizing proteins. They bind to sugar moieties and so are considered to be a type of lectin, cell adhesion proteins that bind sugar polymers.

They are a family of transmembrane molecules and are expressed on the surface of leukocytes and activated endothelial cells. Selectins contain an N-terminal extracellular domain with structural homology to calcium-dependent lectins, followed by a domain homologous to epidermal growth factor, and two to nine consensus repeats (CR) similar to sequences found in complement regulatory proteins. Each of these adhesion receptors is inserted via a hydrophobic transmembrane domain and possesses a short cytoplasmic tail.

There are three subsets of selectins:

- E-selectin (in endothelial cells)
- L-selectin (in leukocytes)
- P-selectin (in platelets and endothelial cells)

L-selectin is the smallest of the vascular selectins, and can be found on most leukocytes. P-selectin, the largest selectin, is expressed on activated platelets and endothelial cells primarily. E-selectin is expressed on activated endothelium with chemically or cytokine-induced inflammation.

The best-characterized ligand for the three selectins is P-selectin glycoprotein ligand-1 (PSGL-1), which is a mucin-type glycoprotein expressed on all white blood cells. Ligands for P-selectin on eosinophils and neutrophils are sialylated, protease-sensitive, endo-beta-galactosidase-resistant structures, and play important roles in recruitment process during inflammatory responses.

Neutrophils and eosinophils bind to E-selectin. One of the reported ligands for E-selectin is the sialylated Lewis X Ag. Eosinophils, like neutrophils, use sialylated, protease-resistant structures to bind to E-selectin.

During an inflammatory response, stimuli such as histamine and thrombin cause endothelial cells to mobilize P-selectin from stores inside the cell to the cell surface. In addition, cytokines such as TNF-alpha stimulate the expression of E-selectin and additional P-selectin a few hours later. The initial attachment of leukocytes, during inflammation, from the blood stream is afforded by the selectin family, and causes a slow downstream movement of leukocytes along the endothelium via transient, reversible, adhesive interactions called leukocyte rolling. i.e. as the leukocyte rolls along the blood vessel wall, the distal lectin-like domain of the selectin binds to certain carbohydrate groups presented on proteins (such as PSGL-1) on the leukocyte, which slows the cell and allows it to leave the blood vessel and enter the site of infection. The low-affinity nature of selectins is what allows the characteristic "rolling" action attributed to leukocytes during the leukocyte adhesion cascade. Each of the three selectins can mediate leukocyte rolling given the appropriate conditions.

IMPORTANT PHYSIOLOGICAL ROLES OF CAMS

Leukocyte-Endothelial Interactions

Under quiescent conditions, neutrophils circulate freely and do not interact significantly with the endothelium. In contrast, monocytes and lymphocytes exhibit a continuous, low-level physiologic traffic across the vessel wall. Monocytes emigrate from the bloodstream to mature into tissue macrophages that may develop tissue- or organ-specific functions. Immune surveillance of tissue requires that lymphocytes recirculate between blood and lymph nodes.

Studies by microscopy have established a sequence of events involved in leukocyte emigration to extravascular sites of inflammation.

Step 1: Leukocyte Trapping or Rolling

The initial, rapidly reversible adhesion of leukocytes to the endothelium under conditions of flow produces rolling, which is the consequence of shear forces acting on the leukocyte and adhesive interactions between selectin receptors and their glycoconjugate counter-structures. Rolling is mediated primarily by the interaction of E and P selectin on activated endothelial cells and leukocyte L selectin with sialylated, fucosylated carbohydrate moieties such as those expressed on various membrane glycoproteins, particularly P selectin glycoprotein ligand-1.

Rolling is initiated primarily by activation of the endothelium by extravascular stimuli such as bacterial derived products or by endogenous mediators produced by the endothelium or tissue cells. Early on, rolling is mediated by endothelial P selectin and leukocyte L selectin. E selectin is involved only at later time points, as it is not constitutively expressed or mobilized but rather is induced over hours by *de novo* synthesis. Leukocyte integrin receptors must be minimally adhesive, in order for them to circulate freely, but able to increase adhesivity rapidly.

Step 2: Leukocyte Activation

Once leukocytes are “tethered” to the endothelium by selectin interactions, the leukocyte integrin receptors are activated by endothelial membrane expressed platelet-activating factor, endothelial membrane bound chemokines, or locally secreted chemoattractants.

Step 3: Leukocyte Activation and Adhesion

Activation of the leukocyte integrins increases their affinity for their endothelial ligands of the immunoglobulin gene superfamily (IgSF). The leukocyte integrins involved in adhesion to endothelial cells are: lymphocyte function antigen-1, macrophage antigen-1, very late antigen. The IgSF ligands are intercellular

adhesion molecule-1 and -2, vascular cell adhesion molecule for very late antigen and the mucosal cell adhesion molecule-1.

Step 4: Diapedesis

Once adherent, the migrating leukocytes move over the endothelial cell surface. On encountering an intercellular junction, some of them then diapedese between endothelial cells to enter extravascular tissue and then migrate to the site of inflammatory or immune reaction. In some vascular beds, leukocytes may emigrate by traversing through, rather than between, endothelial cells. Leukocyte integrin receptor interactions with endothelial IgSF are also likely to be involved in the diapedesis of leukocytes between endothelial cells, whereas subsequent migration through the subendothelial matrix involves the IgSF protein platelet–endothelial cell adhesion molecule. Diapedesis also seems to involve signaling by the leukocyte to the endothelial cell, possibly triggering a disruption of junction integrity.

Step 5: Termination

Leukocyte recruitment is terminated by several mechanisms. E and P selectin are removed from the endothelial cell surface by endocytosis, whereas L selectin is cleaved from leukocytes by a membrane protease. Decay of cytokine, chemokine, or chemoattractant generation leads to gradual resolution of endothelial adhesion molecule expression and integrin activation. Locally generated mediators such as nitric oxide and TGF-β also act to inhibit further leukocyte adhesion to endothelium. This multistep model of selectin dependent tethering and rolling and integrin-dependent firm adhesion and transmigration is supported by observations in gene-targeted mice and in two human leukocyte adhesion deficiency syndromes.

Blood Coagulation and Thrombosis

Under normal conditions, platelets do not adhere to resting endothelial cells, which exhibit multiple, potent antithrombotic properties. After a vascular injury that produces endothelial denudation or retraction, platelets rapidly adhere to the exposed sub-endothelium. The platelets adherent to the damaged vessel wall may directly recruit leukocytes by initiating selectin- and integrin dependent leukocyte adhesion to surface bound platelets. Moreover, the accumulated leukocytes bound to the adherent platelets may promote fibrin deposition, thereby contributing to thrombus formation.

Several platelet receptors have been reported to be involved in the binding to endothelium. Both endothelial sialylated glycoproteins and P selectin on activated platelets have been shown to mediate platelet rolling. Recently, thrombin-activated platelets were shown to bind to endothelial cells by a glycoprotein IIb/ IIIa (integrin αIIbβ3) dependent bridging mechanism involving platelet-bound adhesive proteins, including fibrinogen, fibronectin, and von Willebrand factor. Thus, endothelial adhesion molecules may contribute to the recruitment of activated platelets to intact endothelium and, consequently, to the formation of intravascular platelet aggregates, thereby promoting thrombotic processes (Coller, 1997).

Other studies have shown that mediators such as interleukin-1 and CD40 ligand on activated platelets induce endothelial expression of pro-inflammatory chemokines and adhesion molecules, thereby promoting neutrophil and monocyte adhesion. Adhesion of activated platelets to intact endothelium is therefore also capable of both initiating and amplifying leukocyte recruitment, providing a link between thrombosis and inflammation.

Occlusive thrombus formation in coronary arteries probably begins with the deposition of platelets on a damaged atherosclerotic plaque as a result of the interaction of constitutively active platelet surface receptors [including GPIb/IX, GPIIb/ IIIa (restricted in ligand specificity to immobilized fibrinogen), GPIa/IIa , GPIc/IIa, GPIc/IIa, and perhaps avb3, GPIV, and GPVI] with adhesive proteins in the plaque. The adhesive proteins may be directly exposed by the vascular damage [e.g., collagen or von Willebrand factor (vWf)], deposited from plasma or platelets onto exposed proteins (e.g., the binding of vWf to collagen), or deposited from plasma or platelets onto newly formed fibrin (as for example, vWf). The initial layer of adherent platelets is unlikely to decrease blood flow by itself. Under certain circumstances, however, the platelet GPIIb/IIIa receptors on the luminal surface of the adherent platelets are activated and undergo a conformational change that results in their binding plasma fibrinogen, vWf, or perhaps other glycoproteins with high affinity. The bivalent structure of fibrinogen and the multivalent structure of vWf allow these proteins to bind to GPIIb/IIIa receptors on two different platelets simultaneously. This permits the recruitment of an additional layer of platelets, which in turn can recruit an additional layer of platelets by a similar mechanism, ultimately resulting in vaso-occlusion (Cohen, 1996). In addition, non-occlusive platelet aggregates, if friable, can break off and embolize downstream to small blood vessels, causing both ischemic damage and electrical instability of the heart. The platelet thrombus also facilitates thrombin generation and fibrin deposition, and through exposure of P-selectin, may facilitate leukocyte adhesion and transmigration.

If the GPIIb/IIIa receptor were constitutively in its high affinity ligand binding conformation, thrombosis would be ongoing, and so the receptor is under elaborate control mechanisms that limit its activation both geographically and temporally. Thus, agents that are released (e.g., ADP and serotonin), synthesized and released (e.g., thromboxane A2), or generated as part of the hemostatic cascade (e.g., thrombin) when vessels are damaged are all able to initiate signals that result in

the transformation of the GPIIb/IIIa receptor to a high affinity state. Adhesion itself and shear forces are also able to initiate activation signals, as may thrombolytic agents, either directly or through the paradoxical generation of thrombin.

The currently approved antiplatelet agents, aspirin and ticlopidine, act by inhibiting arachidonic acid metabolism and ADP-induced signal transduction, respectively, but their inhibition of platelet aggregation is incomplete because other pathways can lead to GPIIb/IIIa activation.

Morphogenesis

The ability of cells to detach from a formerly cohesive structure and to migrate and contribute to the formation of new tissues is a key event during morphogenesis (Gumbiner, 1996). A shift from an intercellular mode of adhesion towards a preferential adhesion to an external substrate, via the components of the ECM, is required if active cell migration is to take place. The various cell adhesion molecules referred to in the preceding sections all have a role to play in these processes from a very early stage in development (Edelman, 1985).

Early development

After fertilization and fusion of the sperm and egg pronuclei, the egg divides several times into a mass of cells called a morula. Even at the one cell stage of embryogenesis, there is evidence of E-cadherin expression, and its influence increases with cell compaction. Implantation of the embryo into the uterine epithelium involves both E- and P-cadherin (Takeichi, 1988).

If a monoclonal antibody to E-cadherin is added to the developing morula, the cells detach and further development of the morula is rendered impossible. Under normal circumstances the fertilized egg proceeds from the morula stage to form a blastocyst after 4 days. The blastocyst consists of an outer cell layer called the trophoblast, which develops into the placenta, and an inner cell mass which gives rise to the embryo. By the end of the first week of development a layer of cells known as the hypoblast has formed on the ventral surface of the inner cell mass. The remaining cells of the inner cell mass form the epiblast by the end of the second week of development and the hypoblast and epiblast constitute the bilaminar embryonic disc. These cells maintain the form of the embryonic disc by means of cell-cell and cell-extra-cellular matrix interactions, primarily due to the cell adhesion molecule E cadherin (Viebahn, 1995).

Migration of cells, epithelial-mesenchymal transformation and embryogenesis

The migration of cells is essential for progression of development. Epithelial cells, however, are not noted for their mobility. This is ingeniously corrected by transformation of epithelial cells into mesenchymal cells in a phenomenon known as epithelial mesenchymal transformation (EMT). At an early stage of transformation the epithelial cell down regulates its expression of cell adhesion molecules (in this instance E-cadherin) which frees the attachment of the cells from one another. The cell then enlarges and becomes mobile via its interaction with the ECM. Cell adhesion molecules, particularly integrin, may also be involved at this stage. After migration has been completed the phenotype can again change, with cells reverting back to their epithelial origin in a process predictably called mesenchymal epithelial transformation (MET). The close relationship between EMT and cell adhesion molecules has led some workers to consider the gene controlling E-cadherin as the 'master gene' which switches EMT on and off. The role of EMT in development is considerable. An early example of EMT is the formation of the three germ layers at the gastrulation phase (Hay, 1995).

Tooth development

The C-CAM molecule (a member of the immunoglobulin superfamily of cell adhesion molecules), Syndecan-1 and the ECM glycoprotein tenascin are involved in the first stage of tooth development (dental lamina) and formation of the tooth bud. This interaction continues until the late bud stage and at the cap stage (Kerrigan et al., 1997; Rass et al., 1994).

THERAPEUTIC UTILITY

Ischemic Heart Disease

The rationale for blockade of GPIIb/IIIa receptors as a strategy for preventing or treating ischemic cardiovascular disease rests on data derived from research conducted by many different laboratories: (*a*) platelet thrombus formation secondary to platelet aggregation is the dominant initiating factor in occlusive vascular disease (discussed above); (*b*) the GPIIb/IIIa receptor is a key element in the final common pathway leading to platelet aggregation; (*c*) the GPIIb/IIIa receptor is platelet specific; and (*d*) patients who lack GPIIb/IIIa receptor function on a genetic basis (Glanzmann thrombasthenia) have variably severe mucocutaneous bleeding, but rarely suffer from spontaneous central nervous system hemorrhage (Topol et al., 1999).

Blockade of the GPIIb/IIIa receptor has been accomplished with monoclonal antibodies (C7E3 Fab) or peptides or peptidomimetics based on the RGD (arginine-glycine-aspartic acid) cell recognition sequence; the latter mediates the binding of a number of different ligands to the GPIIb/IIIa receptor and other integrin receptors.

GPIIb/IIIa antagonists are more effective antithrombotic agents than aspirin in a wide variety of animal models. Moreover, when compared with the use of a thrombolytic agent alone, the combination of a GPIIb/IIIa antagonist and a thrombolytic agent produces more rapid and extensive clot lysis, reduces the risk

of re-occlusion, and diminishes infarct size, even when the dose of the thrombolytic agent is reduced by as much as 75%.

Although the predominant antithrombotic benefit of GPIIb/IIIa antagonist therapy most likely results from inhibition of platelet aggregation and thus platelet thrombus formation, additional related phenomena may contribute. For example, platelets probably play an important role in thrombin generation because activated platelets provide a highly efficient catalytic surface that facilitates the reactions leading to thrombin formation and platelets release, and probably activate, Factor V. Thus, GPIIb/IIIa antagonists can potentially decrease thrombin generation via two different mechanisms: quantitatively by decreasing the number of platelets in a thrombus, and qualitatively by decreasing platelet activation and release of Factor V(a). Therefore, decreased thrombin production may contribute to the antithrombotic effects of GPIIb/IIIa antagonists, and thus these antiplatelet agents may also function, in essence, as anticoagulants (Bennett and Mousa, 2001).

Trials of a number of GPIIb/IIIa antagonists in myocardial infarction and unstable angina are currently ongoing in different combinations with aspirin, thrombolytic agents, PCI, stents, and anticoagulants (The EPIC Investigators, 1994; Kereiakes et al., 1996). Other conditions in which platelet thrombus formation may contribute to organ damage may also benefit from GPIIb/IIIa antagonist therapy, including stroke, cerebral and peripheral arterial angioplasty, thrombotic thrombocytopenic purpura/hemolytic uremic syndrome, heparin-induced thrombosis, microvascular surgery, and cerebral malaria. The risks of excessive hemorrhage are considerable, however, in these disorders, and so it is uncertain whether the overall contribution of GPIIb/IIIa receptor blockade will be beneficial.

Three GPIIb/IIIa inhibitors, abciximab, tirofiban, and eptifibatide, have been approved for clinical use in the United States and other countries (Kereiakes, 1999). Also anti-integrin α_v,β_3 blocks human breast cancer growth and angiogenesis in human skin.

Inflammatory Diseases

It is well established that circulating leukocytes are crucial to normal host defense and the immune response, yet under certain conditions they can contribute to host destruction by participation in both acute and chronic immune/inflammatory responses (Sharar et al., 1995). Overzealous accumulation of leukocytes in tissues contributes to a wide variety of diseases, like atherosclerosis, chronic inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, vasculitis, systemic inflammatory response syndrome, juvenile diabetes, psoriasis, etc.

Therapeutic strategies are thus directed to reduce or prevent leukocyte-endothelial cell interactions and communication, in order to limit the progression of inflammatory diseases Blockade of leukocyte adhesion to endothelium by monoclonal antibodies or other antagonists has been demonstrated to reduce vascular and tissue injury in a wide variety of animal models of inflammatory and immune disease. Anti-adhesion therapy directed at lymphocyte trafficking has shown efficacy in several phase 2 and 3 clinical trials in inflammatory bowel disease, multiple sclerosis, and psoriasis (Harlan and Winn, 2002).

Bacterial Diseases

CAMs have also been used by pathogenic microorganisms to evade the immune system (Kerr, 1999). Bacteria generally adhere to the cell lining via CAMs. The advantages of adherent state to bacteria are as follows:

- Required for colonization and for subsequent development of disease (Ofek et al., 2003a)
- Provides significantly greater resistance against clearance by normal cleansing mechanisms, killing by normal immune factors, bacteriolytic enzymes and antibiotics (Ofek and Doyle, 1994)
- Better ability to acquire nutrients, further enhancing ability to survive and infect the host.

Thus the target is to reduce contact between host tissues and pathogens, either by prevention or reversal of adhesion (by use of Anti-adhesive agents) of the infectious agent. This also has an advantage: Anti-adhesive agents are not bactericidal, thus propagation and spread of resistant strains is much less likely to occur than as a result of exposure to bactericidal agents, such as antibiotics (Ofek et al., 2003b).

The major drawbacks are

- Most pathogens possess genes encoding for more than one type of adhesin.
- Involvement of factors other than just adhesin-receptor interactions such as hydrophobic and other non-specific interactions (multiple agents specifically inhibiting each type of adhesin of the infecting pathogen or a single agent that exhibits a broad spectrum of anti-adhesion activity).

Cell adhesion antagonists also have therapeutic potential in asthma and chronic obstructive pulmonary disease as cell adhesion molecules (e.g. selectins and integrins) regulate cell trafficking, regulate leukocyte extravasation, migration within the interstitium, cellular activation, and tissue retention in lungs. Selectins (L-selectin, P-selectin, and E-selectin) and integrins (beta1 and beta2) are thus important therapeutic targets for lung inflammation.

Other Uses

- Anti-integrins or Integrin antagonists (e.g. abciximab, efalizumab, natalizumab) are US FDA-

approved for acute coronary syndromes, psoriasis, and multiple sclerosis, respectively.

- Using monoclonal antibodies directed against WBCs and endothelial cell adhesion molecules may help to limit ischemia-reperfusion injury. Also, blocking leukocyte adhesion to cerebral endothelial cells using a monoclonal antibody to the leukocyte ß-2 integrin adhesion receptor (anti-CD18 MAb) would improve neuronal survival, blood brain barrier (BBB) integrity, and functional outcome following Global Cerebral Ischemia.
- Both active and passive anti-adhesin immunity (Adhesin-based vaccines) can be used to prevent various infections.

Modern proteomics and recombinant biotechnology has helped in the development of unique types of relatively small peptides or adhesion analogs for anti-adhesion therapy eg. synthetic 20-residue peptide mimicking the sequence of a *S.mutans* cell surface adhesion.

Tumorigenesis is another process that involves cell adhesion molecules (Joseph-Silverstein and Silverstein, 1998). For successful tumorigenesis, there must be changes in cellular adhesivity which facilitate the disruption of normal tissue architecture. Additionally, angiogenesis must occur to provide the growing tumor with a blood supply. During metastasis, cells must be able to detach from the primary tumor, enter the blood stream through attachment to a blood vessel wall, travel through the bloodstream, and attach to a vessel wall at a secondary site in order to establish a new tumor.

Defects in cell adhesion molecules are also associated with disease states. For example, leukocyte adhesion deficiency (LAD) syndrome is associated with adhesion cascade defects. LAD I is associated with mutations in the $ß_2$ integrin. There are two forms that have been identified. The first is quite severe, with no LFA-1 ($a_L ß_2$) expression. Patients with the second form, express low levels of $ß_2$ (*i.e.*, about 2 - 5% of normal levels). Patients with the first form of LAD I usually die within a few years of birth unless they receive bone marrow transplantation. Patients expressing the second form of LAD I have a moderate phenotype, but experience numerous types of infections. LAD II results from a defect in the selectins. It is extremely rare and less severe than LAD I. However, patients also exhibit severe mental and growth retardation believed to be due to a generalized defect in fucose metabolism (Etzioni et al., 1999).

RESEARCH

Selectins are involved in significant biomedical research. One project involves using selectins in nanodevices to treat cancer. Researchers are trying to create a device capable of killing cancer cells circulating in the blood. The scientists have covalently attached selectins to an epoxy surface in order to encourage tumor cells and other cells to roll. Also attached to the surface is a ligand that selectively signals cancer cells to undergo apoptosis, or cell death. Without selectins, the device would be unable to slow cancer cells down, and would thus be unable to kill them.

Selectins are also involved in projects to treat osteoporosis, a disease that occurs when bone-creating cells called osteoblasts become too scarce. Osteoblasts develop from stem cells, and scientists hope to eventually be able to treat osteoporosis by adding stem cells to a patient's bone marrow. Researchers have developed a way to use selectins to direct stem cells introduced into the vascular system to the bone marrow. E-selectins are constitutively expressed in the bone marrow, and researchers have shown that tagging stem cells with a certain glycoprotein causes these cells to migrate to the bone marrow. Thus, selectins may someday be essential to a regenerative therapy for osteoporosis.

Study also suggests that plasma cell adhesion molecules may play an important role in the development and progression of peripheral neuropathy in diabetes mellitus (Jude et al., 1998); also the anti-adhesion therapy has found to be useful in multiple sclerosis (Steinman, 2005).

CONCLUSION

The future of anti-adhesion therapy will depend on better knowledge of properties and specificities of various CAMs and on the development of appropriate agents that block adhesion. Once such compounds become available, they can become drugs of choice for the management of a number of diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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