Archives of Nephrology

ISSN: 2639-3573

Volume 2, Issue 2, 2019, PP: 16-28

Role of Cell Adhesion Molecules in Medical Laboratory Diagnosis

Nnodim Johnkennedy*, Diala Tecla Ifesinachi, Ndunelo Chidiebere, Nwajiaku Victor, Nwanebu Chidimma

Department of medical laboratory science, faculty of health science, Imo State University Owerri, Nigeria.

johnkennedy23@yahoo.com

***Corresponding Author:** Nnodim Johnkennedy, Department of medical laboratory science, Faculty of health science, Imo State University Owerri, Imo State, Nigeria.*

Abstract

Cell adhesion is a fascinating process. It plays a key role in many situations of biological and medical interest. It is probably the best cell function to be considered for biophysical modeling from the micrometer to the molecular level. Studying the biophysical aspects of cell adhesion leads to face many important problems of physics and physical chemistry as well as cell physiology. Perturbation of this process leads to the disruption of homeostasis and dysontogenesis. Perturbation of cell-cell adhesion can also be a cause of disease.

Introduction

Cell adhesion is the process by which cells interact and attach to neighboring cells through specialized molecules of the cell surface (Chothia and Jones, 2000). This process can occur either through direct contact between cell surfaces or indirect interaction, where cells attach to surrounding extracellular matrix_ a gel-like structure containing molecules released by cells into spaces between them (Alberts *et al*., 2008). Cell adhesion is crucial for multicellular life. Not only does it allow cells to physically interact with each other and their surroundings to form tissues, but cell adhesion also allows cells to sense their environment and respond accordingly. Therefore, cell adhesion has important roles in immunity, cell migration and embryogenesis (Lessey, 2008). Also tissues have the ability to carry out tasks no single cell could accomplish in its own due to this cell communication. Cell adhesion is also essential for infectious organisms, such as bacteria or viruses, to cause diseases.

Cell adhesion is not just a structural element that binds things together; it is also a highly dynamic process. For example, the cells of the epidermis, called keratinocytes, have to be tightly bound together to provide the barrier properties of the skin that protect humans against fluid loss, infection, and the wear and tear of everyday activities. However, epidermal keratinocytes are constantly being lost from the outer surface and replaced from the basal layer of the multilayered epidermis such that all cells are renewed approximately once every 28 days depending on body site. Other cells such as blood platelets must circulate freely in the blood and thus must be non adhesive. However, when wound occurs, they must become adhesive rapidly to participate in hemostasis, that is, the coagulation of the blood that prevents bleeding.

This important cell adhesion is made possible by the presence of Cell Adhesion Molecules (CAM). Cells express a wide variety of adhesion receptors which may mediate cell-cell interactions, adhesion to the extracellular matrix or both (Alberts *et al*., 2008).

Cell adhesion molecules (CAMs) are proteins located on the cell surface involved in binding with other cells or with the extracellular matrix (ECM) in the process of cell adhesion. In essence, cell adhesion molecules help cells stick to each other and to their surroundings. Cell adhesion is a crucial component in maintaining tissue structure and function. Combined with cell junctions and Extracellular Matrix, CAMs help hold animal cells together (Juliano, 2002).

There are four major families of the CAMs namely: Integrins, Immunoglobulin (Ig) superfamily, Cadherins and Selectins.

Each of these adhesion molecules has a different function and recognizes different ligands. Defects in cell adhesion are usually attributable to defects in expression of CAMs.

History

Recognition of the importance of cell adhesion grew steadily during the twentieth century as it promised answers to fundamental questions in diverse fields that included cell biology, developmental biology, tumorigenesis, immunology and neurobiology.

Early tissue culture studies, initiated by Harrison in 1907, showed convincingly that most tissues were not a syncytium but rather were comprised of individual cells. Thus, the presence of tissues required cell adhesion. The integrity of tissues is also dependent on the adhesion, by CAMs, of cells to cells and cells to the Extracellular Matrix. Studies of tissue dissociation and reassociation in invertebrates also pointed to the importance of adhesion. In 1900, Herbst showed that sea urchin blastomeres fall apart when calcium is removed and then reassociate in normal seawater to form embryo-like structures. Soon after, Wilson observed that sponges could similarly be dissociated into single cells and then reassociate to form a small differentiated sponge (Lewis, 1999).

In 1975, Gerald Maurice Edelman discovered substances called cell adhesion molecules (CAMs), which "glue" cells together to form tissues. Edelman found that, as the brain develops, CAMs bind neurons together to form the brain's basic circuitry. His work led to the construction of a general theory of brain development and function called neuronal group selection.

Structure and Interaction of Cell Adhesion Molecules

CAMs are typically transmembrane receptors and are composed of three conserved domains: an intracellular domain that interacts with the cytoskeleton, a transmembrane domain, and an extracellular domain (Chothia and Jones, 2000).

These proteins can interact in several different ways which could be hemophilic or heterophilic protein-protein interactions or protein-carbohydrate interactions.

In Homophilic interactions, CAMs bind with the same CAMs on another cell as seen in Cadherins and Immunoglobulins Superfamiliy. Such homophilic binding leads to selective adhesion between cells of the same type.

For example, nerve Cell Adhesion Molecules (N-CAMs) are members of the Ig superfamily expressed on nerve cells, and homophilic binding between N-CAMs contributes to the formation of selective associations between nerve cells during development (Park *et al*., 2010).

Cadherins form homophilic attachment between themselves, which results in cells of a similar type sticking together and can lead to selective cell adhesion, allowing vertebrate cells to assemble into organized tissues. Cadherins are essential for cell–cell adhesion and cell signaling in multicellular animals (Chothia and Jones, 2000).

In heterophilic binding, a CAM on one cell will bind with different CAMs on another cell or the extracellular matrix as seen in Integrins and Selectins.

In multicellular organisms, bindings between CAMs allow cells to adhere to one another and creates structures called cell junctions.

Classification of the Cell Adhesion Molecules

These four families of the Cell Adhesion Molecules are categorized based on their Calcium dependence.

Integrins and the Ig-superfamily CAMs do not depend on Ca 2+ while cadherins and selectins depend on Ca 2+ (Alberts *et al*., 2008)

Integrins

Integrins are large, membrane-spanning, hetero-dimeric proteins (as they consist of an alpha and beta subunit) that are essential for a metazoan existence. All members of the integrin family are transmembrane proteins found in all animal cells that bind to extracellular matrix ligands, cell-surface ligands, and soluble ligands, thus they facilitate Cell-ECM Adhesion (Humphries, 2000). Even simple animals like sponges have these proteins. Integrins are among the major classes of receptors within the Extracellular matrix. Integrins provide essential links between the extracellular environment and the intracellular signaling pathways, which can play roles in cell behaviors such as apoptosis, differentiation, survival, and transcription. The presence of Integrins allows rapid and flexible responses to events at the cell surface (Alberts *et al*., 2008).

Structure of the Integrins

Integrins are obligate heterodimers, meaning that they have two subunits: α (alpha) and β (beta) linked together by disulphide bond.

There are currently 18 alpha subunits and 8 beta subunits, which combine differently to make up 24 different receptors with different binding properties and different tissue distribution. Variation in the alpha and beta subunits accounts for the wide variety of integrins observed throughout the animal kingdom. For example, humans alone have over 20 different kinds of integrins. Within each of the alpha and beta subunits there is a large extracellular domain, a transmembrane domain and a short cytoplasmic domain.

Integrin subunits span the cell membrane and have short cytoplasmic domains of 40–70 amino acids. The exception is the beta-4 subunit, which has a cytoplasmic domain of 1,088 amino acids, one of the largest of any membrane protein (Humphries, 2000).

Outside the cell membrane, the alpha and beta chains lie close together along a length of about 23 nm; the final 5 nm N-termini of each chain forms a ligand-binding region for the ECM. They have been compared to lobster claws, although they don't actually "pinch" their ligand, they chemically interact with it at the insides of the "tips" of their "pinchers".

The molecular mass of the integrin subunits can vary from 90 kDa to 160 kDa. Beta subunits have four cysteine-rich repeated sequences. Both alpha and beta subunits bind several divalent cations. The role of divalent cations in the alpha subunit is unknown, but may stabilize the folds of the protein. The cations in the beta subunits are more interesting: they are directly involved in coordinating some of the ligands that integrins bind (Giancotti and Ruoslanti, 2010).

Integrins can be categorized in multiple ways. For example, some α chains have an additional structural element (or "domain") inserted toward the N-terminal; the alpha-A domain (so called because it has a similar structure to the A-domains found in the protein von Willebrand factor), it is also termed the alpha-I domain. Integrins carrying this domain either bind to collagens (e.g. integrins α-1 β-1, and α-2 β-1), or act as cell-cell adhesion molecules (integrins of the β-2 family). This α-I domain is the binding site for ligands of such integrins. Those integrins that don't carry this inserted domain also have an A-domain in their ligand binding site, but this A-domain is found on the β- subunit (Humphries, 2000).

In both cases, the A-domains carry up to three divalent cation binding sites. One is permanently occupied in physiological concentrations of divalent cations, and carries either a calcium or magnesium ion, the principal divalent cations in blood at median concentrations of 1.4 mM (calcium) and 0.8 mM (magnesium). The other two sites become occupied by cations when ligands bind—at least for those ligands involving an acidic amino acid in their interaction sites. An acidic amino acid features in the integrin-interaction site of many ECM proteins, for example as part of the amino acid sequence Arginine-Glycine-Aspartic acid (Giancotti and Ruoslanti, 2010).

Functions of the Integrins

Integrins have two main functions:-

I. Attachment of cell to the ECM and Cell Migration

II. Signal transduction from the ECM to the cell

However, they are also involved in a wide range of other biological activities, including immune patrolling, and binding to cells by certain viruses, such as adenovirus, echovirus, hantavirus, and foot-and-mouth disease viruses.

Attachment of Cell to the ECM and Cell Migration

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 defined by which alpha (α) and beta (β) subunits the integrin is made of. Among the ligands of integrins are fibronectin, vitronectin, collagen, and laminin. 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 (Juliano, 2002).

The attachment of the cell takes place through formation of cell adhesion complexes, 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. Integrin α6β4

is an exception: it links to the keratin intermediate filament system in epithelial cells (Frisch and Screaton, 2011).

One important function of integrins on cells in tissue culture 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. It is not yet clear whether cell migration in tissue culture is an artifact of integrin processing, or whether such integrin-dependent cell migration also occurs in living organisms (Jacquemet *et al.,* 2013)

Signal Transduction

Signal transduction is the process by which a chemical or physical signal is transmitted through a cell as a series of molecular event, most commonly by protein phosphorylation catalyzed by protein kinases, which ultimately results in a cellular response. Proteins responsible for detecting stimuli are generally termed receptors or sensors (Frisch and Screaton,2011).

Signal transduction by Integrins is bidirectional meaning that they can transmit information both outside-in and inside-out (Hynes, 2012).

Integrins play an important role in cell signaling by modulating the cell signaling pathways of transmembrane protein kinases such as Receptor Tyrosine Kinases (RTK). Integrins can regulate the receptor tyrosine kinase signaling by recruiting specific adaptors to the plasma membrane. For example, β1c integrin recruits Gab1/Shp2 and presents Shp2 to IGF1R, resulting in dephosphorylation of the receptor. In a reverse direction, when a receptor tyrosine kinase is activated, integrins co-localise at focal adhesion with the receptor tyrosine kinases and their associated signaling molecules. (Focal adhesions are large molecular complexes, which are generated following interaction of integrins with ECM, then their clustering). Clustering and activation of the integrins/actin complexes strengthen the focal adhesion interaction and initiate the framework for cell signaling through assembly of adhesomes (McGray *et al*., 2012).

Depending on the integrin's regulatory impact on specific receptor tyrosine kinases, the cell can experience;

- celll growth
- cell division
- cell survival
- cellular differentiation, and
- apoptosis (programmed cell death)

Some cell signaling occurs on a local level, such as when cells interact with the surrounding extracellular matrix or with their immediate neighbors. This type of signaling is especially important to the structure and function of tissues. Various signaling molecules allow the cells within a tissue to share information about internal and external conditions. This information helps the cells arrange themselves, coordinate their functions, and even know when to grow and when to die.

It is worthy to also note that Integrins have an important function in neuroregeneration after injury of the peripheral nervous system (PNS).

A prominent function of the integrins is also seen in the molecule GPIIbIIIa, an integrin on the surface of blood platelet (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 collagens in the wound site. Upon association of platelets with collagen, GPIIbIIIa changes shape, allowing it to bind to fibrin and other blood components (McGray *et al*., 2012).

Immunoglobulin Superfamily

The immunoglobulin superfamily (IgSF) cell adhesion molecules are large calcium-independent transmembrane glycoproteins that are involved in the recognition, binding, or adhesion processes of cells (Barclay, 2003).

Molecules are categorized as members of this superfamily based on shared structural features with immunoglobulins (also known as antibodies). They all possess a domain known as an immunoglobulin domain or fold. The purpose of immunoglobulins (antibodies) is to recognize and adhere to other molecules.

IgSF has over 765 members, and is one of the largest and most diverse families of proteins in the body (Barclay,2003).

Members of the Ig superfamily include;

- Vascular Cell Adhesion Molecules (VCAM)
- Neural Cell Adhesion Molecules (NCAM)
- Intercellular Adhesion Molecules (ICAM)
- Nectin and nectin-like (Nec1)family.

Over 50 different members of the immunoglobulin superfamily are found to be expressed in the mammalian nervous system. The extracellular domain of IgSF CAMs typically mediate Homophilic cell adhesion, i.e., cell adhesion mediated by interactions between the same molecules in membranes of adjacent cells.

They are commonly associated with roles in the immune system. Otherwise, the sperm-specific protein IZUMO1, a member of the immunoglobulin superfamily, has also been identified as the only sperm membrane protein essential for sperm-egg fusion (Juliano, 2002).

Structure of the Immunoglobulin Superfamily

Proteins which have a similar structure to immunoglobulin are classified as IgSF. Each IgSF CAM has an extracellular domain, which contains several Ig-like intra-chain disulfide-bonded loops with conserved cysteine residues, a transmembrane domain, and an intracellular domain that interacts with the cytoskeleton (Halaby and Mornon, 2015).

The Immunoglobulin Domains

Proteins of the IgSF possess a structural domain known as an immunoglobulin (Ig) domain, this Immunoglobulin (Ig) domain is the common feature of immunoglobulin superfamily members.

Ig domains are named after the immunoglobulin molecules. They contain about 70-110 amino acids and are categorized according to their size and function. Ig-domains possess a characteristic Ig-fold , which has a sandwich-like structure formed by two sheets of opposing antiparallel beta strands stabilized by a disulphide bridge - Interactions between hydrophobic amino acids on the inner side of the sandwich and the highly conserved disulfide bonds formed between cysteine residues in the B and F strands, stabilize the Ig-fold. One end of the Ig domain has a section called the complementarity determining region that is important for the specificity of antibodies for their ligands (Barclay, 2003).

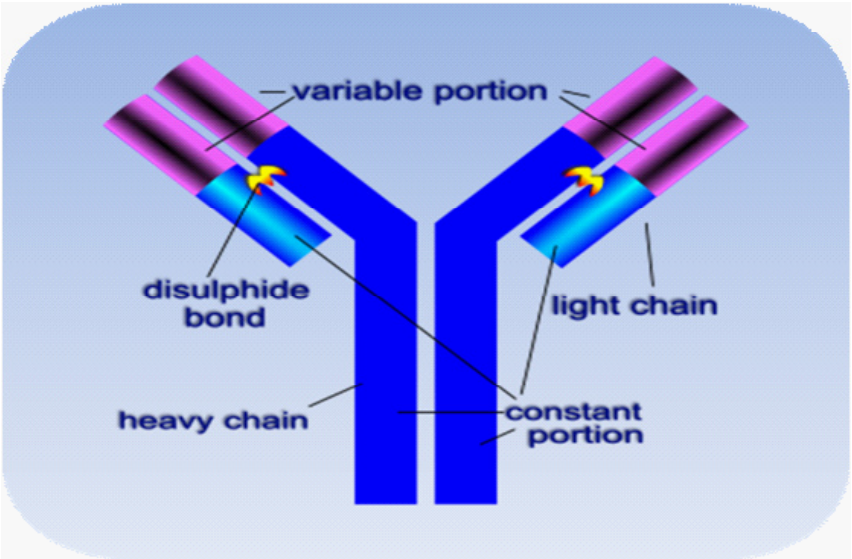

Fig 1. *Diagram representing structure of the Immunoglobulin Domain of the IgSF.*

Source: Halaby and Mornon, 2015.

Classification of the Immunoglobulin Superfamily

Classified based on the Immunoglobin domains. Most Ig domains are either variable (IgV) or constant (IgC) and thus could be classified as:

I. IgV,
II. IgC1, IgC2 or
III. IgI

IgV

IgV domains with have 9 beta strands are generally longer than IgC domains with 7 beta strands.

IgC1 and IgC2

IgC2 are similar in size to IgC domains but resemble IgV domains in the amino sequence. While standard IgC domains are called IgC1 domains.

IgI

Other Ig domains exist that are called intermediate (I) domains (Halaby and Mornon, 2015).

Functions of the IGSF

IgSF CAMs play key role in the developing nervous system by regulating migration of neurons, growth and branching of axons and dendrites and establishment of contacts between neurons (Maness and Schachner, 2007).

Immunoglobulin superfamily also play central roles in regulating adaptive and innate immune responses, and are prime targets for the development of protein-based therapeutics.

Cadherins

Cadherins are a class of cell adhesion molecules that are dependent on Ca2+ ions for their function. They are known as class-1 transmembrane 120 kD glycoprotein that mediate cell-cell adhesion in animals. By regulating contact formation and stability, cadherins play a crucial role in tissue morphogenesis and homeostasis. Cadherins function in tissue morphogenesis by controlling both cell-cell adhesion and cell signaling and holding the tissues together as they form during embryonic development.

Cell-cell adhesion is mediated by extracellular cadherin domains in the presence of calcium whereas the intracellular cytoplasmic tail associates with a large number of adaptor and signaling proteins, including linkers to the cytoskeletal network. Loss of function of cadherins has also been linked to cancer, and some researchers are investigating cadherins as drug targets (Angst *et al*., 2011).

Cadherin Structure

Cadherins are named for calcium dependent adhesion. Cadherins are synthesized as polypeptides and undergo many post-translational modifications to become the proteins which mediate cell-cell adhesion and recognition. These polypeptides are approximately 720-750 amino acids long. Each cadherin has a small cytoplasmic component, a transmembrane component- piece that penetrates through the membrane and the remaining bulk of the protein is extra-cellular (outside the cell).

The external domain of a cadherin molecule - the part that is on the outside of a cell - is made up of many repeats of the same protein chain. Classical cadherins have five cadherin repeats. Each repeat has a space for binding calcium. Calcium makes the chain rigid, helping it to connect with a chain from another cell (Angst *et al.,* 2011).

Most cadherins adhere by homophilic interaction (i.e. they bind to the same type of cadherin) but certain types (e.g. E-cadherin) also adhere by heterophilic interactions (i.e. they bind other type of cadherin). Cadherin association is sensitive to extracellular calcium hence their name, calcium adhering (Juliano, 2002). The interactions can take place laterally on the same cell, called a cis interaction or between two cells, called a trans interaction (Gumbiner, 2005).

The functionality of cadherins relies upon the formation of two identical subunits, known as homodimers. The homodimeric cadherins create cell-cell adhesion with cadherins present in the membranes of other cells through changing conformation from cis -dimers to trans-dimers. Once the cell-cell adhesion between cadherins present in the cell membranes of two different cells has formed, adherens junctions can then be made when protein complexes, usually composed of α-, β-, and γ-catenins , bind to the actin cytoskeleton portion of the cadherin. It should be noted that individual cadherin interactions are weak. The strength of cadherin-based adhesive junctions comes from the clustering of multiple, weak cadherin-cadherin interactions, thus, although cadherin-cadherin binding between the extracellular domains is relatively weak, the conformational changes that are induced after binding imparts the individual cadherins with rigidity. This stabilizes the interaction and fosters additional lateral cis interactions with other cadherins and generates tighter adhesions. Increased clustering of cadherins at sites of cell-cell contact correlate with increased stability and maturation of actin-based structures such as dendritic spines (Beavon, 2011).

Types of Cadherins

There are about one hundred types of cadherins in vertebrates, and they fall into four groups; Classical, desmosomal, protocadherins, and unconventional. The classical cadherins can also be further sub-divided

in to several categories based on their location and function (Hulpiau, 2009). Invertebrates contain fewer than 20 types of cadherins.

Classical Cadherins

The classical cadherins are the most common family members. The Cadherins are transmembrane proteins, and the N and C terminal of the cadherins are present in the extracellular and intracellular domain of a cell, respectively. Classical cadherins consist of five cadherin domains which are termed EC1-EC5 (Hulpiau, 2009).

The classical cadherins can also be further sub-divided into several categories based on their location and function, namely:

i) E-Cadherin (Epithelial): This cadherin is present in epithelial cells, hence the name 'E-Cadherin. Defects in E-Cadherin adhesion have been associated with several diseases, such as cancer, Listeriosis, Candidiasis, Bacteroides infection.

ii) N-Cadherin (Neural): This cadherin is present in neurons, fibroblasts, and muscle cells, hence the name, N-Cadherin. Defects in N-Cadherin are associated with cancer (skin, prostate, pancreatic and gastric) and Candidiasis.

iii) P-cadherin (placental): P-cadherins are found in the placenta.

Desmosomal Cadherins

Desmosomes are intercellular cadherins that connect intermediate filaments and cardiac muscle. They are important in forming a type of cellular junction called a desmosome. It includes:

- Desmoglein(Dsg) and
- Desmocollin (Dcg).

Dsc and Dsg are approximately 30% identical to each other in terms of amino acid sequence.

Desmocollins are present in epidermis, hair follicle cells, myocardium, stratified epithelia while Desmogleins are present in simple and stratified epithelia, myocardium, hair follicle cells, and the outermost epidermis, among other tissues.

Proto-Cadherins

Protocadherins are a large group of cadherin molecules present in a wide range of species that are thought to be related to an ancestral cadherin. Their extracellular domains have more than five repeated cadherin motifs, distinguishing them from classical cadherins. The intracellular domains of the protocadherins are also different from their classical cousins. They are highly variable, with a variety of functions in the nervous system, including neuronal differentiation and the formation of synapses.

Unconventional/Ungrouped Cadherins

Ungrouped cadherins are a large group of cadherins that are not otherwise categorized into the previous three groups. They include VE-cadherin, R-cadherin, and many others (Hulpiau, 2009).

Function of the Cadhedrins

Cadherins' Special Role in Cancer

E-cadherin, a member of the cadherin family, has a very important role in organizing the epithelium. Most cancers arise from epithelial tissue, and in those cancers, cell adhesion mediated by E-cadherin is lost at the same time that a tumor progresses toward malignancy. Some studies have focused on restoring E-cadherin's function in the epithelium as a mechanism for cancer therapy (Nguyen, 2014).

Select in

Selectins are a group of cell adhesion glycoprotein that play a key role in the initial immunological response. It is a group of cell surface molecules that influence the attachment and movement of white blood cells to other cells and to the lining of blood vessels (the first adhesive step during inflammation and immune surveillance), e.g., in inflammatory diseases and conditions. It is specialized in capturing leukocytes from the bloodstream to the blood vessel wall. The migration of leukocytes from the blood vessel into inflamed tissue is the central step in the process of inflammation. The tethering of leukocytes to the endothelial cell surface is mediated by selectins (Ley, 2013).

All selectins are single-chain transmembrane glycoproteins that share similar properties to C-type lectins due to a related amino terminus and calcium-dependent binding. The absence of selectins or their ligands has severe health consequences, with recurrent bacterial infections and persistent disease occurring. Due to their essential role in leukocyte recruitment, selectins are an attractive target for

therapeutic intervention against diseases involving excessive inflammatory response as found in numerous cardiovascular and autoimmune diseases (McGray *et al*., 2012).

Structure of the Selectins.

All three known members of the selectin family (L-, E-, and P-selectin) share a similar cassette structure:

- an N-terminal, calcium-dependent lectin domain,
- an epidermal growth factor (EGF)-like domain,
- a variable number of consensus repeat units (2, 6, and 9 for L-, E-, and P-selectin, respectively),
- a transmembrane domain (TM) and
- an intracellular cytoplasmic tail (cyto) (Chothia,2000).

Types of Selectins

There are three types of selectins according to the cell type in which it was first characterized:

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

L-selectin is the smallest of the vascular selectins, expressed on all granulocytes and monocytes and on most lymphocytes, can be found in most leukocytes. P-selectin, the largest selectin, is stored in α-granules of platelets and in Weibel–Palade bodies of endothelial cells, and is translocated to the cell surface of activated endothelial cells and platelets. E-selectin is not expressed under baseline conditions, except in skin microvessels, but is rapidly induced by inflammatory cytokines (Chothia,2000).

Roles of the Selectin.

Selectins have been implicated in several roles but they are especially important in the immune system by helping white blood cell homing and trafficking and also, they play very essential role in Human Implantation (Ley, 2013).

Role in Immune System

Selectins are required for the trafficking of innate immune system cells, T lymphocytes and platelets. The absence of selectins or their ligands has severe health consequences, with recurrent bacterial infections and persistent disease occurring. During inflammation, selectins enable the initial attachment of leukocytes from the bloodstream, which causes their downstream movement along the endothelium via adhesive interactions referred to as leukocyte rolling (Ley, 2013).

Role in Human Implantation

In the last decades, Selectin and it's ligands have been found to play an essential role in the process of human implantation (Liu *et al*., 2011).

For a successful implantation process, the appropriate arrival time, a viable blastocyst and a receptive endometrium are the three essential elements (Norwitz *et al.,* 2010).

Furthermore, the attachment at the embryo-endometrium contact surface is the key step in starting a successful implantation process. From a morphological point of view, there is a remarkable resemblance between vascular leucocyte extravasation and attachment of the embryo to the endometrium. L-selectin expressed on the surface of the trophoblast and L-selectin ligand expressed on the surface of the endometrial epithelium play crucial roles in the adhesion of the blastocyst and endometrial epithelium. After the trophoblast has successfully attached to the endometrial epithelium, there is now a diversion of maternal blood to the placenta (Carter *et al.,* 2015).

Relevance of Cell Adhesion Molecules in Medical Laboratory Diagnosis

Cell adhesion molecules have been recognized to play a major role in a variety of physiological and pathological phenomena and advances in the treatment of human diseases rely upon the understanding of the patho-physiology of the disease process.

Role in Cancer

Dysfunction of cell adhesion occurs during cancer metastasis. Loss of cell-cell adhesion in metastatic tumor cells allows them to escape their site of origin and spread through the circulatory system (Friedl, 2003). Indeed, changes in the expression or function of cell adhesion molecules have been implicated in all steps of tumor progression, including detachment of tumor cells from the primary site, intravasation into the blood stream, extravasation into distant target organs, and formation of the secondary lesions (Johnson, 1999). One example of CAMs deregulated in cancer are cadherins, which are inactivated either

by genetic mutations or by other oncogenic signaling molecules, allowing cancer cells to migrate and be more invasive, endowing on the neoplastic cells an invasive and migratory phenotype. This contributes to cancer invasion and metastasis. Alterations in the expression of E- Cadherin have been found in esophageal carcinoma, gastric carcinoma, breast Cancer, bladder carcinoma and head and neck carcinomas (Nguyen, 2014).

Other CAMs, like selectins and integrins, can facilitate metastasis by mediating cell-cell interactions between migrating metastatic tumour cells in the circulatory system with endothelial cells of other distant tissues.

Due to the link between CAMs and cancer metastasis, these molecules could be potential therapeutic targets for cancer treatment (Chiang and Massagué, 2008).

Leukocyte Adhesion Deficiency -I (Lad-I) Disease

This is a genetic disorder which causes the immune system to malfunction, resulting in a form of immunodeficiency. Patients with LAD-1 have an inherited molecular defect that causes a deficiency of the β-2 integrin subunit, also called CD18, which is encoded by the ITGB2 gene found on chromosome 21. This subunit is involved in the formation of the β-2 integrins, thus expression of the β-2 integrin subunit is reduced or lost. The main function of these proteins is to allow neutrophils to make their way out of the blood stream to the infected tissues by adhering to different ligands expressed by the endothelium, e.g. ICAM-1. In LAD-1 patients, neutrophils cannot extravasate and fight against bacteria in tissues. The bacteria can then proliferate, leading to symptomatic infection, which can spread unimpeded and cause serious injury to important tissues (Robert *et al.,* 2012).

Starting from birth, people with leukocyte adhesion deficiency type 1 develop serious bacterial and fungal infections. One of the first signs of leukocyte adhesion deficiency type 1 is a delay in the detachment of the umbilical cord stump after birth. In newborns, the stump normally falls off within the first two weeks of life; but, in infants with leukocyte adhesion deficiency type-1, this separation usually occurs at three weeks or later. In addition, affected infants often have inflammation of the umbilical cord stump (omphalitis) due to a bacterial infection. These individuals do not form abscesses because granulocytes cannot migrate to the sites of infection (Medkaikar *et al.,* 2012).

In the treatment of this disease, although patients can receive intensive antibiotherapy and even granulocyte transfusions from healthy donors, the only current curative therapy is the hematopoietic stem cell transplant. However, gene therapy is also effective (Robert *et al.*, 2012).

As a Predictive Marker of Human Uterine Receptivity

L-selectin ligand can be used clinically as a marker for implantation efficiency (Lessey, 2008).

Human blastocysts utilize L-selectin to initiate implantation by binding to endometrial ligands composed of oligosaccharide moieties on the surface glycoproteins. The absence of these ligands could lead to recurrent implantation failure (RIF).

Altered expression of Selectins and their ligands has been found to be associated with abnormal pregnancies and infertility (Liu *et al.,* 2011).

Patients with RIF (repeated lack of implantation after the transfer of embryos) tested for the presence of the L-selectin ligands by immunohistochemistry usually shows the absence, or much reduced presence of the L-selectin ligand as finding has proved that L-selectin plays a role in implantation, not only by its presence, but also by its degree (Norwitz *et al.,* 2010).

Screening for the absence of the ligand may help many patients with RIF to avoid undergoing repeated failed treatment cycles.

Pemphigus

Pemphigus is an autoimmune blistering skin disease as it results from autoantibodies targeting a person's own desmosomal cadherins which leads to epidermal cells detaching from each other and causing skin blistering (Yeh *et al.,* 2013).

In pemphigus, keratinocytes in epidermis and mucous membranes lose cell-cell adhesion, a process called acantholysis. IgG autoantibodies are characteristically directed against desmogleins (desmoglein-1 and desmoglein-3), which are part of the cadherin family of cell-cell adhesion molecules that are found in desmosomes, which are the structures primarily responsible for maintaining intercellular adhesion in stratified squamous epithelia, such as the skin and oral mucosa.

Circulating anti-Dsg antibodies are found in pemphigus (Beavon,2014).

The primary treatment modality has been oral corticosteroids (which are used as an anti-inflammatory agent because of their suppressive effects on the inflammatory and allergic responses). In addition, several immuno suppressants, including azathioprine, cyclosporine, mizoribine, mycophenolate mofetil and cyclophosphamide, are used (Yeh *et al*., 2013).

Fig 2. *Image of a palm infected with Pemphigus*

Source: Yeh *et al*.,2013

Role in Infection

Pathogenic microorganisms, including bacteria, viruses and protozoans, have to first adhere to host cells in order to infect and cause diseases and this is made possible by adhesion molecules.

Protozoans express multiple adhesion molecules with different specificities that bind to carbohydrates located on surfaces of their host cells. An example of a pathogenic protozoan is the malarial parasite (Plasmodium falciparum), which uses one adhesion molecule called the circumsporozoite protein to bind to liver cells, and another adhesion molecule called the merozoite surface protein to bind red blood cells. Pathogenic fungi use adhesion molecules present on its cell wall to attach, either through protein-protein or protein-carbohydrate interactions, to host cells or fibronectins in the extracellular matrix. Viruses also have adhesion molecules required for viral binding to host cells. For example, influenza virus has a hemagglutinin on its surface that is required for recognition of the sugar sialic acid on host cell surface molecules. HIV has an adhesion molecule termed gp120 that binds to its ligand CD4 , which is expressed on lymphocytes. Viruses can also target components of cell junctions to enter host cells, which is what happens when the hepatitis C virus targets occludins and claudins in tight junctions to enter liver cells (Mateo *et al* .,2015).

Anti-adhesion therapy can be used to prevent infection by targeting adhesion molecules either on the pathogen or on the host cell to alter their production. Apart from altering the production of adhesion molecules, competitive inhibitors that bind to adhesion molecules to prevent binding between cells and pathogenic organisms, can also be used, acting as anti-adhesive agents (Ley, 2013).

Research

Selectins are 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 (Ley, 2013).

Critical Illness Polyneuropathy

In cases of elevated blood glucose levels, as in diabetes, also in sepsis, plasma cell adhesion molecules are higher than normal especially E-selectin resulting in greater microvascular permeability. The greater permeability leads to edema (swelling) of the skeletal endothelium (blood vessel linings), resulting in skeletal muscle ischemia (restricted blood supply) and eventually necrosis (cell death). This underlying pathology is the cause of the symptomatic disease, Critical Illness Polyneuropathy (CIP) (Jia *et al.,* 2001).

Increased concentrations of adhesion molecules have also been associated with multiple organ dysfunction, disease severity, or death.

Laboratory Diagnosis of the Cell Adhesion Molecules

Blood level of soluble form of cell adhesion molecules can be effectively diagnosed in the laboratory using Enzyme Linked ImmunoSorbent Assay (ELISA) Technique.

Specimen types: Serum, Heparin Plasma

Specificity: This assay has high sensitivity and excellent specificity for detection of human CAMs. No significant cross-reactivity or interference between human CAMs and analogues has being observed.

Assay Type: Sandwich

Detection Range: 0. 156 ng/ml -10 ng/ml. Thus, its sensitivity is very high.

Principle of the Assay: This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific to the Cell Adhesion Molecule is immobilized on the surface of microplate wells and incubated first with the target CAM and then with another target antigen-specific antibody, which is labeled with an enzyme. This target antigen will then be recognized and bound by the detection antibody conjugated to biotin and streptavidin-HRP. After washing, the enzyme substrate is added, and the activity of the microplate well-bound enzyme is measured by the colour development, which is proportional to the amount of CAMs bound in the initial step.

The immobilized antibody and the enzyme-labeled antibody must recognize different epitopes of the target protein.

Procedure

Antibody specific for CAMs is been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CAMs present is bound by the immobilized antibody.

After removing any unbound substances by washing, a biotin-conjugated antibody specific for CAMs is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells.

Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of CAMs bound in the initial step. The color development is stopped and the intensity of the color is measured.

Conclusion

Cell adhesion molecules are critical to many normal physiological processes. Given their widespread importance it is not surprising that cell adhesion molecules have also been implicated in many diverse pathological processes such as inflammation and wound healing, septic shock, cancer, and atherosclerosis.

Recently, an understanding of the role of cell adhesion molecules in these processes has suggested their use as either diagnostic or prognostic markers, or as potential targets for therapeutic intervention. This is best exemplified in cancer

References

[1] Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K and Walter, P. (2008). Molecular Biology of Cell. *Nature Review Molecular Cell Biology*. 359(6):2952-2923.

[2] Angst, B., Marcozzi, C and Magee, A. (2011). The Cadherin Superfamily: Diversity in Form and Function. *Journal Cell Science*. 114(4):629-641.

[3] Barclay, A, N. (2003). Membrane proteins with immunoglobulin-like domains—a master super family of interaction molecules. *Seminars in Immunology.* 15(4):215-223.

[4] Beavon, I.R. (2014). The E-Cadherin-Catenin complex in tumour metastasis: Structure, function and regulation. *European Journal of Cancer.*36(13):1607-1620.

[5] Bergom, C., Gao, C and Newman, P.J. (2005). Mechanisms of PECAM-1-mediated cytoprotection and implications for cancer cell survival. *Leukemia and Lymphoma.* 46(10):1409-1421.

[6] Carter, A. M., Enders, A.C and Pijnenborg, R. (2015). The role of Invasive trophoblast in implantation and placentation in primates. *Biology Science*. 370(1663): 2014-2070.

[7] Chiang, A.C and Massagué, J. (2008). Molecular basis of metastasis. *The New England Journal of Medicine.* 359(26):2752-2823.

[8] Chothia, C and Jones, E.Y. (2000). The molecular structure of cell adhesion molecules. *Annual Revised Biochemistry*. 66:823-862.

[9] Frisch, S.M and Screaton, R.A. (2011). Anoikis Mechanism. *Current Opinion in Cell Biology*. 13:555-562.

[10] Friedl, P and Wolf, K. (2003). Tumour-cell invasion and migration: diversity and escape mechanisms. *Nature Reviews Cancer*. 3(5):362–374.

[11] Giancotti, F. G and Ruoslanti, E. (2010). Integrin Signalling.*Science.* 285(5430):1028-1032

[12] Gratzinger, D., Barreuther, M and Madri, J.A. (2003). Platelet-endothelial cell adhesion molecule-1 modulates endothelial migration through its immunoreceptor tyrosine-based inhibitory motif. *Biochemical and Biophysical Research Communications.* 301(1):243-249.

[13] Gumbiner, B.M.(2005). Regulation of Cadherin-mediated Adhesion in Morphogenesis. *Nature Review Molecular cell biology*. 6(8):622-634.

[14] Halaby, D.M and Mornon, J. P. (2015). The immunoglobulin superfamily: an insight on its tissular, species and functional diversity. *Journal of Molecular Evolution.* 46(4):389-400.

[15] Hulpiau, P and VanRoy, F. (2009). Molecular evolution of the Cadherin Superfamily. *International Journal Biochemistry Cell Biology.* 41(2):349- 369.

[16] Humphries, M.J. (2000). Integrin Structure. *Biochemical Society Transactions.*28(4):311-339.

[17] Hynes, R. (2012). Integrins: bidirectional, allosteric signalling machines. *Cell.*10(6):673-687.

[18] Jacquemet G., Humphries, M. J and Caswell, P. T. (2013). Role of adhesion receptor trafficking in 3D cell migration. *Current Opinion in Cell Biology*. 25(5): 627-632.

[19] Jia, W., Wang, R., Zhao, J., Lou, I.Y., Zhang, D., Wang, X and Han, X. (2001). E-Selectin expression increases in human cerebral aneurysm tissues. *The Canadian Journal of Neurological Sciences.* 38(6): 858-862.

[20] Johnson, J.P. (1999). Cell adhesion molecules in the development and progression of malignant melanoma. *Cancer and Metastasis Reviews*. 18(3):345–357.

[21] Juliano, R.L. (2002). Signal transduction by cell adhesion receptors and the cytoskeleton: functions of integrins, cadherins, selectins, and immunoglobulin-superfamily members. *Annual Review of Pharmacology and Toxicology.* 42:283-323.

[22] Lessey, B. A. (2008). Adhesion Molecules and Implementation. *Journal of Reproduction Immunology.* 55:101-112.

[23] Lewis, W.H. (1999). The adhesive quality of cells. *Anatomy Record.*23:387–398.

[24] Ley, K. (2013). The role of Selectins in inflammation and disease. *Trends in Molecular Medicine.* 9(6):263-280.

[25] Liu, S., Yang, X., Liu, Y., Nang, X and Yan, Q. (2011). L-selectin Mediates adhesion in vitro Implantation model. *Molecular Cell Biology*. 350(1):185- 192.

[26] Maness, P.F and Schachner, M. (2007). Neural recognition molecules of the immunoglobulin superfamily: signaling transducers of axon guidance and neuronal migration. *National Neuroscience*. 10:19-26.

[27] Martin, A.C., Gelbart, M., Fernandez-Gonzalez, R., Kaschube, M and Wieschaus, E. F. (2013). Integration of contractile forces during tissue invagination. *Journal of Cell Biology*. 188: 735-749.

[28] Mateo, M., Generous, A., Sinn, P.L and Cattaneo, R. (2015). Connections matter how viruses use cell cell adhesion components. *Journal of Cell Science*. 128(3):431-439.

[29] McGary, E.C., Lev, D.C and Bar-Eli, M. (2012). Cellular adhesion pathways and metastatic potential of human melanoma. *Cancer Biology*. 5:459-465.

[30] Medkaikar, M., Currimbhoy, Z., Gupta, M., Desai, M and Rao, M. (2012). Clinical profile of leukocyte adhesion deficiency type 1. *Indian Pediatrics*. 49(1):43-45

[31] Mehlen, P and Puisieux, A. (2006). Metastasis: a question of life or death. *Nature Reviews Cancer.* 6(6):449-458.

[32] Nguyen, T. H. (2014). Mechanisms of metastasis. *Clinics in Dermatology*. 22(3): 209-216.

[33] Norwitz, E.R., Schust, D. J and Fisher, S. J. (2010). Implantation and the survival of early Pregnancy. *New England Journal of Medicine*. 342:1400-1408.

[34] Park, S., DiMaio, T.A., Scheef, E.A., Sorenson, C.M and Sheibani, N. (2010). PECAM-1 regulates proangiogenic properties of endothelial cells through modulation of cell-cell and cell-matrix interactions. *American Journal of Physiology-Cell Physiology*. 299(6):1468-1484.

[35] Robert, P., Canult M., Farnarier C., Nurden A., Grosdidier C., Barlogis V and Alessi M.C. (2012). A Novel Leukocyte Adhesion Deficiency 1 Variant. *Journal of Immunology*. 186(9):5273-5283.

[36] Sahai, E. (2007). Illuminating the metastatic process. *Nature Reviews Cancer*. 7(10):737-749.

[37] Yeh, S.W., Ahmed, B., Sami, N and Ahmed, A.R. (2013). Blistering disorders: diagnosis and treatment. *Dermatologic Therapy*. 16(3)214-230

Citation: **Nnodim Johnkennedy, Diala Tecla Ifesinachi, Ndunelo Chidiebere, Nwajiaku Victor, Nwanebu Chidimma.** *Role of Cell Adhesion Molecules in Medical Laboratory Diagnosis. Archives of Nephrology. 2019; 2 (2): 16-28.*