C E L L A D H E S I O N

In multicellular organisms, cells form tissues for specialized functions. For such high degree of organization it is indispensable for a connection to be established between cells as well as between cells and elements of the Extra Cellular Matrix (ECM). Besides the connections maintaining a strict tissue structure, these interconnections also play an important role in cell migration, cell differentiation and communication between cells. Both the cell adhesion and cell-matrix connections are results of dynamically changing interactions established by ECM components (such as collagen, glycosaminoglycans, proteoglycans, fibronectin, laminin), plasma membrane-associated cell surface adhesion proteins, and occasionally the anchoring molecules between cytoskeletal elements and membrane proteins.

The aim of the consultation is:
During the consultation the student shall:

1) Familiarize him/her-self with certain classes of adhesion molecules as well as their structural and functional characteristics.

2) Learn the biological role of cell adhesion molecules.

3) Interpret on a molecular level the transformation of connections between cells in two important pathological states (in tumor development and leukocyte migration during inflammation).

Cell surface adhesion molecules

When treating a sample of different tissue types with special proteolytic enzymes, cells will dissociate from each other. In such circumstances, scientists have observed how cells, despite being mixed together with dissimilar cell types will coalesce and reestablish a connection with cells of the same tissue origin. More interestingly, even in cases where cells were taken from different species and treated in the same manner as before, cells tended to "stick" and adhere to cells of the same tissue type. The explanation for this can be found in the properties of the cell adhesion molecules (CAM), which are evolutionary conservative molecules whose expression on the cell surface depends on the given cell type and the cell's current status (e.g. resting or activated, degree of differentiation).

A so called "homotype" connection is established between two cells using the same type of cell-adhesion molecule, while a "heterotype" connection is established when adhesion takes place between two structurally different proteins. Although the actual physical interaction between adhesion molecules is generally considered "weak", the great number of additional near lying connecting type of proteins ensures an appropriate level of cohesion (known as the "zipper" principle). Usually proteins do not independently establish a connection, but instead organize in groups, as part of a greater cell adhesion structure (or complex).

Traditionally cell adhesion interactions have been grouped into Ca^{2+} dependent and Ca^{2+} independent classes depending on whether a connection takes place in the presence or absence of Ca^{2+}. More recently we categorize cell adhesion molecules based on their protein structure: 1) Integrins 2) Cadherins 3) Cell adhesion molecules belonging to the superfamily group of immunoglobulins 4) Selectins.

Integrins

Integrins are responsible for the anchoring of cells to the ECM, cell to cell interactions and also two-way signal transmission, since they take part in pathways that carry signals both to and from the cell. These properties make the integrins suitable for such complex mechanisms as blood coagulation, inflammation, migration, tissue differentiation and cell division.

Heterodimeric structural transmembrane proteins that require bivalent cations for their operation; usually calcium ions. In mammals, 24 different integrin molecules result from a combination of different α and β subunits, found in various cell types. Each cell can carry a variety of different integrins. Although some integrins are specific because of their ligand-selectivity, (e.g. only bind fibronectin or laminin), integrins can usually bind many different ligands. Most integrins bind to *RGD (arginine-glycine-aspartate)* amino acid sequences. This tripeptide is not only found in ECM macromolecules (collagen, laminin, fibronectin, vitronectin), but also in plasma (soluble fibronectin, fibrinogen, von Willebrand factor) and in cell surface proteins (e.g. in a number of hormone and neurotransmitter receptors; which is also a possible adhesive function research subject). The cytoplasmic domain of the integrin molecule links to actin filaments via talin, vinculin, α-actinin, filamin and beta1-integrin-linked protein kinase (ILK) connecting proteins. The integrin of hemidesmosomes connects intracellularly to intermediate filaments.

The *Mac1* ($\alpha_M\beta_2$-integrin) works as an intercellular adhesion molecule. Mainly present on macrophages, this integrin actually functions as a complement receptor. Its ligand is the cell-bound C3b complement; recognized as a foreign fragment. The effect of ligand binding is phagocytosis by macrophages of what the immune system considers harmful and of the complement fragment-marked (opsonized) cell. Mac1 along with LFA also takes part in leukocyte adhesion.

The *LFA1* ($\alpha_L\beta_2$-integrin) is expressed on leukocytes. Its ligands are ICAM1 and ICAM2, members of the immunoglobulin superfamily. Its main task is to anchor the leukocytes rolling along the vessel wall to the endothelial surface. This adhesion allows for the extravasation and emigration of white blood cells, thus forming the localized tissue inflammatory reaction. The hereditary LFA1 defect leads to the disease known as Type 1 leukocyte adhesion deficiency (LAD1), (see below).

The glycoprotein known as *Gp IIb/IIIa* ($\alpha_{11b}\beta_3$-integrin) is present on platelet membranes. Following platelet activation, it undergoes a conformational change and the RGD binding site moves to the surface for the binding of blood coagulation proteins (fibrinogen, von Willebrand factor). This is the basis for the process of platelet aggregation. In clinics, Gp IIb/IIIa antagonists of coagulation are becoming increasingly common in the treatment and prevention of abnormal activation of blood coagulation (e.g. thrombosis). The hereditary protein defect in Gp IIb/IIIa is associated with Glanzmann's type thrombasthenia, a coagulopathy with prolonged bleeding time.

Cellular junctional complexes (focal contacts, hemidesmosomes) made up mostly of integrins are discussed below.

Cadherins

Within the superfamily of cadherins, exist one or more transmembrane sections of glycoproteins, on whose extracellular domains repetitive cadherin-repeat subdomains can be found. Calcium binds to these repetitive protein sequences. The subsequent conformational change allows bond formation between the cadherin molecules of neighboring cells. Cadherins are therefore calcium-dependent, „homotypic" contact-establishing adhesion molecules. The cadherins can connect with the cytoskeleton's actin and intermediate filaments on the cytoplasmic side of the plasma membrane through protein complexes. These protein complexes are organized around the cadherin molecules' intracellular domains. It is thus understandable, that cadherins take part in a number of signal transduction pathways.

The cadherins that were first discovered were named after the tissue in which they were found. *E-cadherin* is responsible for epithelial cell cohesion, for example, it can be found in the skin, intestinal epithelium, kidney tubules and plays an important role during embryonic morphogenesis. Also, the main component of zonula adherens is *E-cadherin* (see below:cell adhesion structures). *N-cadherin* is an important adhesion protein of neuronal and lens cells. It also plays a role in the integrity of muscle fibers and in the intercellular connections of heart muscle fibers (fascia adherens). *P-cadherin* holds together placental trophoblast cells, but can also be found in the epidermis, intestinal epithelium, the heart and the lungs.

The *VE-cadherin* coupler of vascular endothelial cells plays an important role in vascular permeability, leukocyte trans-endothelial migration and in angiogenesis. The latter function is responsible for bringing this molecule to the forefront of cancer research. Other, less common cadherins: *M-cadherin* of myocytes and *R-cadherin* of retinal neurons and the heart. The *T-cadherin* does not have a transmembrane section; using the membrane anchor glycosylphosphatidylinositol. It is not an adhesion protein yet it plays an important role in the pathomechanism of atherosclerosis with the uptake of LDL particles.

Adhesion molecules of the Immunoglobulin Superfamily (IgSF CAM)

The immunoglobulin superfamily contains a number of cell surface and soluble proteins, which are involved in cell recognition, binding, stabilization and communication (involving growth factors and cytokine receptors). Their common structural element is the immunoglobulin domain which was first discovered via antibodies produced by plasma cells. The members of the cell adhesion superfamily adhere to each other by way of the immunoglobulin domains extending from the membrane. Identical cell to cell bonds are thereby created (homotypic binding) yet they are also able to bind to other cell surface molecules as well (heterotypic binding). Both types of binding are calcium- independent.

N-CAM was one of the superfamily's first described molecule. As it turned out, the N-CAM of neurons is not actually one molecule, but a family of molecules, coded for by the same gene. Alternative splicing in the transcription on mRNA results in differently sized protein products. Post-translational glycolysation of proteins results in polysialic acidcarbohydrate chains, which because of negative repulsive interactions, weaken the homophilic bonds between N-CAM molecules. This explains why high sialic acid-content N-CAMs on embryonic neurons are replaced by low-sialic acid content molecules by adulthood; since during development transient and easily disrupted cell to cell connections, rather than strong bonds leading to stabile tissue structures are more desirable. According to recent studies, importance has been given to N-CAM polysialic sequences in learning and in memory (neuronal plasticity) as well. Aside from neurons, N-CAMs can be found in glial cells, NK cells and in striated muscle fibers.

The *ICAM* (intercellular adhesion molecule) group of proteins are mainly expressed in endothelial and white blood cells. The created heterophilic bonds' most important partner is the integrin $\alpha_L\beta_2$ (LFA1), expressed solely on white blood cells. A hereditary defect in the $\alpha L\beta 2$-integrin results in the leukocyte adhesion deficiency (LAD) disease (see below). Within the immune response, ICAM proteins are involved in several processes, not just in the extravasation of white blood cells. Examples of ICAM protein involvement include antigen presentation and the initiation of T cell proliferation.

Several types of immunoglobulin adhesion molecules following cytokine stimulation appear on the endothel surface only after activation. The ligands of *VCAM1* are similar to the white blood cell integrins of the ICAM group and are also responsible for the occurrence of leukocyte adhesion. Recently, the role of VCAM1 and its potential for therapeutic blocking has been recognized as beneficial for various disease processes. Melanoma cells are capable of using the proteins of

extravasation from within the vessel, which is an important element in the process of metastasis. Similarly, the development of metastasis from colon tumors to the liver can be attributed to VCAM1.

Selectins

Selectins have a number of transmembrane domains, and link themselves to the carbohydrate groups of neighboring cell surfaces. The calcium-dependent binding of the protein molecule concludes with the key role played by the *lectin domain*. The resulting bond is heterophilic: the selectin binds to the carbohydrate side chains of other cells' surface proteins or lipids; and even more specifically to the different oligosaccharide sequences. We distinguish three different types of selectins.

E-selectin can be found on endothelial cells; their expression dependent on cytokine stimulation (i.e. IL-1, TNFα). Its ligands are the surface sialic and fucose-containing glycoproteins on the different white blood cells (monocytes, granulocytes, T-lymphocytes). The resulting bond is weak, the white blood cells are not statically fixed to the endothelium. With these bonds, the white blood cells have the ability to travel in the same direction of blood flow; but much more slowly along the vessel wall („rolling"). Another effect of stimulation is the activation of the rolling leukocytes and their extravasation through the endothelium. All of these processes are important in the inflammatory mechanisms.

P-selectins are displayed on endothelial cells only minutes after a stimulus. In contrast to E-selectins, these molecules are not newly synthesized, but arise from intracellular stores---emerging from Weibel-Palade bodies to the cell surface. Although the P-selectin has similar physiological functions to E-selectins, (white blood cell „rolling"); it differs in ligands (with the same carbohydrate sequences, but different protein components) and in stimulus-causing agents (primarily histamine and thrombin). P-selectins can also be found in the α-granules of platelets, and from here they reach the surface following thrombocyte activation.

The *L-selectin* of leukocytes acts as a „lymphocyte homing" receptor, also sharing the previously mentioned role of slowing down the white blood cells. Its ligands are the glycosylated membrane proteins of endothelial layers that are found in vessels spanning lymphoid tissue; for example, the GlyCAM1 gylcoprotein ligand expressed on endothel cells of HEVs (high endothelial venules) in lymph nodes.

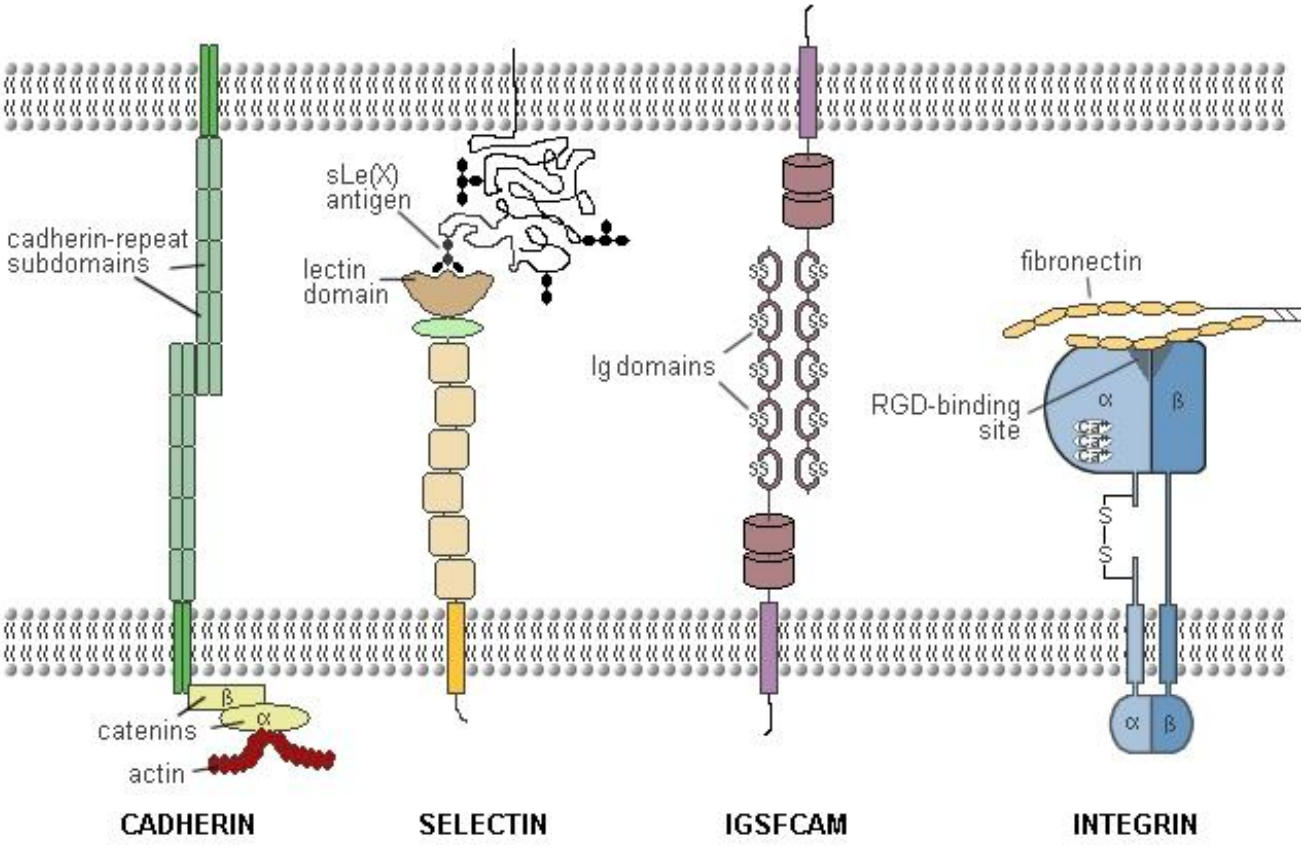
The ligand-binding of selectins is mediated by fucose of the glycan side chain. The fucose enters the Golgi apparatus in its GDP-fucose form, where fucosyl-transferase enzymes bind it to the already synthetized glycoproteins– which will later reach the cell surface. The missense mutation of the enzyme that transports GDP-fucose from the cytosol to the Golgi is responsible for Leukocyte adhesion deficiency type 2 (LAD2), (see below).

Cell-matrix adhesion proteins

We discussed the main cell surface receptor family involved in ensuring the cell-ECM contacts: the integrins. The most important ligands are those matrix proteins (fibronectin, vitronectin, collagen, laminin) that contain binding sites for both ECM fibers and integrins projecting from the cell membrane, equally. With this, they glue together the extracellular fiber system to the cell surface proteins, and through cell surface proteins to the cytoskeleton. This binding is essential for the maintenance of tissue structure stability. Furthermore, the above mentioned proteins are important for cell migration, forming routes („tracks") for the movement of cells. Through cell migration, cells of neural tube origin are able to move to the periphery during embryonic development; and fibroblasts and keratinocytes can migrate to areas of damage in wound healing.

Fibronectin is a dimer composed of two polypeptide chains linked by two disulfide bridges. It carries binding sites for collagen, heparin, heparin-sufate, the fibrin formed at the end of the coagulation cascade, transmembrane proteoglycans and to an extent for integrins (which is the previously mentioned RGD or Arg-Gly-Asp sequence). Alternative splicing of the fibronectin gene results in two different proteins. The first of these proteins, the *cell surface fibronectin* often polymerizes into fibers, the fibers then mesh together into a network. Such a network stabilizes the routes used by macrophages and tissue regenerative cells during cell migration. The other protein is primarily produced in the liver and circulates in the blood in its soluble form: soluble or *plasma fibronectin*. Following mRNA splicing, the exon responsible for polymerization is removed, therefore plasma fibronectin does not become polymerized. With vascular damage, collagen is exposed and fibronectin binds; undergoing a conformational change. As a result, the affinity of fibronectin for the activated GpIIb/IIIa platelet integrin greatly increases.

I. Figure Cell adhesion molecules



From this follows its most important function: platelet aggregation, and thereby promoting a stable clot formation.

Laminin binds the cells to the basal lamina. The basal lamina is not only found under the epithelial- and endothel layers but also around other non-epithelial type cells. Aside from laminin, it contains type IV collagen, heparin-sulfate and nidogen. The binding site of laminin is associated with the other basal lamina components or with cell surface proteins (integrins and other glycoproteins). A

hereditary defect of laminin results in severe skin disorders, muscular dystrophy and disruptions in glomerular filtration.

Cellular junctional complexes

Epithelial cells attach to themselves and to the extracellular matrix through junctional complexes. The supramolecular organizations allow the cells to maintain their mechanical connections, collect information on the cell's environment, relay electrical and chemical signals to other cells, as well as restrict the diffusion of solutes in the lateral intercellular space ("barrier" function).

Mechanical connections provide junctions that may be tight (*zonula occludens*, or **tight junctions**), adhesive contacts (*zonula adherens*, or **adherens junctions**), desmosomal connections (*macula adherens*, or **desmosome**); as well as *focal adhesions* and *hemidesmosomes* that connect the cells to the ECM. The transmission of electrical and chemical signals is facilitated by a specialized intercellular connection, known as a **gap junction** or nexus. Junctional complexes mediating adhesion and communication between adjacent and contacting cells secure tissue differentiation and later provides mechanical strength. Since the proteins of such coupling structures are apart of signal transduction processes, their function cannot be restricted to only maintaining mechanical connections. Specific signaling proteins travel between adhesion sites and the cell nucleus.

Zonula occludens (tight junctions, TJ)

Typically, adjacent plasma membranes of epithelial cells are held together by sealing strands. These strands are composed of transmembrane proteins that make contact across the intercellular space to create a seal. Tight junctions create the "barrier" that is selectively permeable to certain substances. *Occludin*, the *claudin*s and *JAM* (junctional adhesion molecule) tighten neighboring epithelial cells and adaptor proteins (ZO-1, 2, 3; symplekin, cingulin); connecting them to the microfilament system of the cytoskeleton. The laterally arranged protein complexes are closely packed and create a branching network of sealing strands that completely encircle the apical parts of the cells.

An important task of the zonula occludens is to prevent the free migration of membrane components (such as integral transport proteins) between the apical and basolateral membranes; allowing for the specialized function of each surface. An example for this "fence" function is the active transport of substances from the intestine. Important substances needed for the body from the bowel are absorbed in two steps. First, with the aid of the transport proteins located on the apical membrane, the substances enter the epithelial cell. From there, they move through the basolateral side of the cell into the interstitial space, and finally into the bloodstream. The transport moving in the direction of: lumen ----> intestinal epithelial cell, ----> extracellular space (interstitium) is carried out by carrier proteins that are significantly different from each other. They are specific to the transported molecule and its concentration.

There are two types of mechanisms by which molecules or pathogens can pass through an epithelial cell. One is the so-called *paracellular transport* between adjacent cells that may also allow for the breaking of cellular junctions. The second is the *transcellular transport*, whereby a molecule/particle enters the cell by endocytosis, passes through and exits by exocytosis. (Most recently this process has been called transcytosis.)

Paracellular transport, which is always passive diffusion, is essential for providing a system that is capable of preventing backflow of the substances which have been absorbed into the interstitium

back into the bowel lumen. The zonula occludens connection is so strong, that even with aid of an electron microscope, the intercellular gap cannot be separated from the connecting membrane areas (hence the name: tight junctions); and is capable of effectively blocking the rediffusion of molecules. This barrier characteristic to prevent rediffusion of molecules, is the second function of tight junctions in the uptake of nutrients from the bowel lumen. Tight junctions are impermeable to macromolecules, but certain small water-soluble molecules and ions may pass from the lumen into the interstitial space (and of course, vice versa); probably through putative aqueous pores within the claudin based barrier of the tight junction strands. This paracellular transport allows for the passive diffusion of substances present in high concentrations in the lumen to be absorbed (e.g. amino acids).

The recently discovered zonula occludens toxin (Zot) is an enterotoxin, and known as the causative agent of cholera, product of the bacteria Vibrio cholerae. It induces a reversible opening of the zonula occludens. The toxin only binds to certain parts (jejunum and terminal ileum) of the intestinal epithelial cells where the permeability is already larger than what is found in other intestinal segments.

An example of transcellular transport is the Shiga toxin, which exerts its effects on endothelial cells, but it must reach the epithelial tissue in order to be absorbed. The TLR receptor (toll-like receptor) mediated transcellular transport is its most recognized mechanism.

The junctional complex, zonula occludens, is not only found in renal tubules and intestinal epithelium, but also in the blood-brain barrier; providing a dynamic interface between the peripheral circulation and the central nervous system. The structure of the zonula occludens connecting the endothel cells of the blood-brain barrier is very similar to that of the previously mentioned epithelial cells. Its main function is to inhibit the paracellular diffusion of polar solutions from the blood plasma into the brain tissue. Aside from the brain capillary endothelial cells, the paracellular permeability of the blood-brain barrier is also affected by astrocytes and pericytes.

Tight junctions in cerebral endothelial cells are composed of the same transmembrane proteins as found in epithelial cells: the claudins, occludin and JAM; but these are tissue-specific isoforms. Paracellular permeability strongly depends on the integrity of the TJ complex, which is regulated by the reversible phosphorylation of proteins.

Generally, when tight junction proteins shift from the apical part of the cell into the interior, or when the expression of TJ proteins decrease, there is a resultant increase in paracellular permeability. This may occur, for example, in cases of hypoxia (induced either by high altitude e.g. climbing or pathological conditions such as stroke). During cerebral ischemia the zonula occludens proteins (claudin-5 and occludin) of brain capillary endothelial cells delocalize from the membrane and their expression becomes reduced--thereby increasing the paracellular permeability. In addition, the localization of the ZO-1, -2 adaptor proteins (scaffolding molecules, linking both the zonula occludens and zonula adherens to the cytoskeleton thereby providing stability) also changes; they delocalize from the membrane to the nucleus, inducing the expression of transcription factors, (Fos, Jun).

Zonula adherens

The zonula adherens is a belt-like structure that runs around the apical part of cells and is found directly below the zonula occludens. As long as the zonula occludens is primarily responsible for the inhibition of paracellular transport, the zonula adherens serves to localize and stabilize the zonula occludens. It can be found in almost all tissues, for example, between the connections of myocytes in the myocardium (fascia adherens of the intercalated discs); or in many other tissues, such as in the synapses of the CNS. The cell to cell contact of this structure is ensured by the cadherin molecules in the presence of Ca^{2+} ions bound to the extracellular domains. E-cadherin is characteristic in epithelial cells, while N-cadherin is involved in muscle cells and in the fascia adherens. The zonula adherens is made up of actin filaments running in a ring-like manner along its cytoplasmic side and is anchored by

protein complexes. It is the α-, β-, γ-catenins and vinculin protein attached to the transmembrane cadherins on the inner surface of the membrane that stabilize it to the actin ring. The actin bundles are fixed by α-actinin molecules. The zonula adherens is, overall, a coherent contractile network, formed by the cells' adherent actin rings. This contractile structure was shown to be important in morphogenesis, in the development of tubular structures creating a lumen.

The zonula occludens and adherens are very closely situated to each other in the apical region of the lateral membrane. The zonula adherens maintains the integrity of the tight junctions, so together the two junctions create the apical junctional complex. Some components of the apical junctional complex belong to the so-called PDZ family of proteins. That is, they contain a domain (PDZ) that recognizes the C-terminal four amino acid PDZ binding site of other proteins. Thus, a protein network is formed that contributes to maintaining cellular polarity.

The importance of PDZ domain-containing proteins has been shown in experiments where the proteins were specific targets for certain virus oncoproteins, and the virus PDZ protein interactions led to cancerous lesions. Such an example is the human papilloma virus, (HPV), that attacks the epithelial PDZ proteins, causing the cell to lose polarity and simultaneously causing density independent proliferation, cell cycle disruption and tissue lesions.

Desmosomes

Desmosomes are spot-like structures that connect two neighboring cells. Under an electron microscope they appear as dark, dense structures on the cytoplasmic side of the membrane. To this plate-like disc, that is formed by *desmoplakin* and *γ-catenin* (formerly known as plakoglobin), attaches the intermediate filaments of the cytoskeleton. The cadherin molecules *desmoglein* and *desmocollin* are also anchored to these desmosomes, as they protrude from the membrane towards the neighboring cell. Although the Ca^{2+} dependent, homotypic relationship between the desmosomal cadherins in itself is weak, the large number of bonds together form a serious, cohesive ensemble. The significance of this is clear when one considers that desmosomes keep together the keratinocytes of the epidermis, therefore they must be resistant against all types of influences. Outside the epidermis, we can find desmosomal contacts in the capillary endothelium, the lymphatic system, or the intercalated discs in the myocardium, for example. The epidermal blistering disease *pemphigus*, autoantibodies are produced against desmoglein and bind to desmosomes. The antibody-binding induces an immune reaction, after which the destruction of desmosomes leads to the separation of epithelial cells from each other, followed by a build-up of fluid in these areas.

While the defect in desmoplakin primarily involves the skin , a γ-catenin (plakoglobin) gene defect leads to hereditary autosomal recessive Naxos disease (or hereditary arrhythmogenic right ventricular dysplasia). This disease is characterized by palmar and plantar keratoderma, woolly hair, and right ventricular fatty-fibrotic change with subsequent arrhythmias.

Focal adhesions

Focal adhesions are junctions connecting cells to the ECM. Integrins are responsible for their adhesive property and connect to the intracellular actin filaments: *talin, vinculin, paxillin* and *α-aktinin.* The mature focal adhesion is formed from over 150 proteins. Aside from providing matrix stability, the proteins can dynamically connect and disconnect according to the cells needs; allowing for cell migration. In addition, this junction between the cell and its environment is also involved in two-way signaling. We can find focal adhesions in muscle cells, keratinocytes, fibrocytes, and white blood cells.

Many other adaptor proteins also connect to the focal contact complex, for example, the kindlins. A hereditary mutation in one of the members of this protein group may lead to Kindler syndrome. This disease is characterized by extreme fragility and blistering of the subepidermis (part of the epidermolysis bullosa disease group). Some members of the kindlin family may also occur around E-cadherins (zonula adherens) and associate with the nuclear membrane.

Cell migration

During cell migration, the cells **polarize** in the direction of motion. The cell will develop a leading edge (lamellipodia) and a tail section (uropod). Cell migration is a multistep, cyclical process, that can be broken down into several steps: (1) following polarization of the cell, it will extend its leading edge towards the direction of movement (2) new cell adhesions will form between the cell and matrix (focal complexes) (3) the actin network develops stress fibers (4) contraction of actomyosin fibers causes the cytoplasm of the tail section to flow in the direction of the leading edge (5) the mature focal adhesions on the tail end will disintegrate while simultaneously new focal adhesion complexes will emerge Cells may migrate independently (individual cell migration) or within groups (cluster migration). In cluster migration, the cells retain their connection structures.

Hemidesmosomes

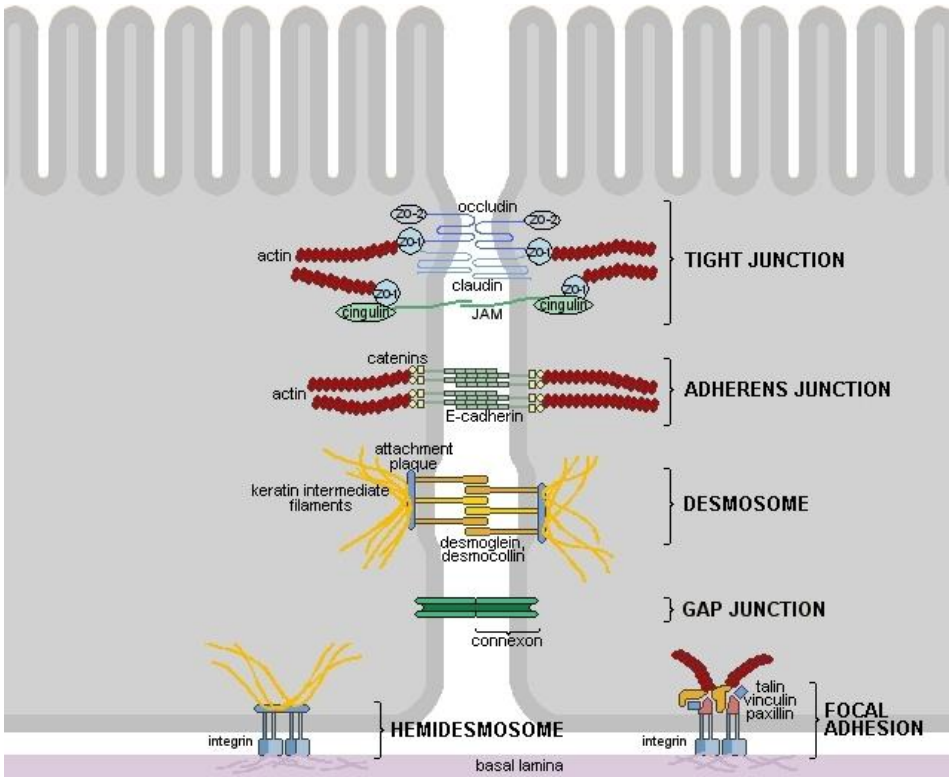
Primarily occuring in the dermis-epidermal border of the skin, the role of hemidesmosomes is to *stabilize the basal keratinocyte layer to the basal lamina*. Under electron microscopy, its appearance is similar to that of a halved desmosome, however they are significantly different in their construction. The integrins anchored to cytoplasmic plaques connect to the basal lamina collagen through laminin proteins. Intracellularly, *keratin* intermediate filaments connect to these plaques.

The transmembrane collagen XVII also takes part in the composition of the hemidesmosome protein complex. The N-terminal portion of the hemidesmosome connects to the homotrimer protein of its cytoplasmic plaque, thereby giving stability to the structure. In a congenital defect or in conditions where autoantibodies are produced against this protein, a skin disease with blistering may occur.

Electrical coupling (nexus, gap junction)

These junctions are formed as a channel protein complex between two adjacent cells. Based on the diameter of this channel, molecules greater than 2000 Da cannot cross. The main function of electrical coupling is *signal transduction*; the passive diffusion of permeable molecules are mostly second messengers (e.g. cAMP, Ca^{2+}). A specific response in the channel is evoked according to the type of molecule passing though, for example, exocytosis or contraction. Another important function of electrical coupling is the coordination of metabolism. In tissues with poor blood supply such as the eye lens or distal parts of bone, for example, this coupling mechanism ensures that cells are met with an adequate supply of nutrients. Its significance continues in embryogenesis, where in addition to nutritional requirements, it is essential that a certain group of cells respond uniformly to a stimulus. Gap junctions are found between neurons, thereby enabling the direct excitation transfer to occur as a unity of synapses. Here, ions are transferred along an electrochemical gradient, giving the effect that is not any different from a postsynaptic cell depolarization. The main advantage of the electrical synapse over a chemical synapse is speed; while the main disadvantage is the bidirectional transfer. The depolarizing potential may be conducted through muscle cells (myocytes, smooth muscle cells) simultaneously with synchronized contraction (which also allows for the presence of gap junctions, e.g. around intercalated discs or between gastrointestinal smooth muscle cells).

II. Figure Cell adhesion structures in the intestinal epithelium



The actual channel (gap junction) between two adjacent cells is composed of *connexon* units, which are inherently related to the intercellular gap. The connexon unit itself is comprised of 6 *connexin* subunits flanked together to create an internal pore diameter of 1.2-nm. The permeability of the gap junction is regulated by the intracellular ion composition. In the event of cell damage and subsequent abnormal elevations of intracellular Ca^{2+} ion concentration, the gap junction will close. This mechanism serves to protect neighboring cells while a similar mode of regulation also protects the cell from extreme changes in intracellular pH values.

Mutations of connexin subunits have been found in the background of numerous congenital (inborn) diseases. For example, mutations of the Cx-26 protein, most abundantly expressed in the cochlea, leads to neurosensory hearing impairment, which is presumably related to the inappropriate distribution of K^+ ions within the fluid of the inner ear. A defect in the Cx-32 isoform protein is responsible for a certain kind of neuropathy and muscle dystrophy called Charcot-Maire-Tooth syndrome. The disease begins with muscle atrophy of the lower limbs, presumably around the nodes of Ranvier and Schmidt-Lantermann gaps; which leads to Schwann cell degeneration as the diffusion paths supplying nutrition to the cells have been compromised because of gap junction injury

Adhesion molecules in the formation of tumor metastasis

The development and formation of cell adhesion connections, their maintenance and termination are all important events in tumor progression and metastasis. Adhesion molecules play an equal part in the detachment of cells from a tumor mass, their migration and settlement in distant tissue as well as in angiogenesis. In order to illustrate the steps involved in metastatic transformation an example of a malignant colorectal carcinoma (CRC) is discussed on a basic molecular level which has spread to the liver. The underlying events are similar if not identical for all malignant tumors. The details and mechanics related to lymphogenic metastasis are much less well understood.

The first step is the detachment of the tumor cell from the tumor mass. Normally, when epithelial cells lose their connection to their immediate environment (in other words, they no longer adhere to their surrounding ECM) apoptotic cell death occurs (the phenomenon is called *anoikis,* a Greek word meaning "statelessness"). However, malignantly transformed cells are able to avoid apoptosis and remain viable. First these cells regain their migratory capacity (a required condition), and then breakthrough the vessel wall. A precondition for this ability is for the cells to produce matrix metalloproteases. Then the cells either individually or as a group manage to pass between the endothelial cells. The detached cell enters the blood circulation (or is already there, in case the tumor had previously infiltrated the vessel) and through the smaller intestinal veins ends up drifting into the portal veins. Hepatic colonization by tumor cells begins with invading the sinusoids, where they come into contact and adhere to sinusoidal endothelial cells, Kupffer cells and Ito cells. Successful anchoring to the sinusoidal endothel makes extravasation and proteolytic penetration into the liver parenchyma possible. In case the tumor cell in the parenchyma survives and starts to replicate, a secondary tumor develops (metastasis), whereby every penetrating cell will be genetically identical and be considered a clone (unless a genetic mutation takes place among the replicating secondary tumor cells). During tumor growth neither the primary nor the metastatic tumor can function without adequate oxygen and nutritional supply. For this reason, when the diameter exceeds the maximal distance for simple diffusion of nutrients, the tumor induces new vessel generation (angiogenesis).

Among the *immunoglobulin superfamily* of adhesion proteins, *ICAM1* and *VCAM1* appear to be important from the viewpoint of metastasis. Based on experiments with rats it appears that the CRC cells induce liver Kupffer cells to produce inflammatory cytokines (e.g. TNF-α), which in turn increase the expression of ICAM1 and VCAM1 on sinusoidal endothelial cells. These adhesion proteins thereby bind tumor cells which are then able to leave the blood stream. It is not known precisely which factor induces the Kupffer cells to produce the above mentioned cytokines, but is most likely due to the antigens *CEA* (carcinoembryonal antigen) on the surface of malignantly transformed intestinal epithelial cells. Clinically, this tumor marker molecule belongs to the immunoglobulin superfamily of proteins. The role of the immunoglobulin domain containing adhesion molecules is thus on two levels: partly the CEA-CEA-receptor (on Kupffer cells) attachment initiates Kupffer cells' cytokine secretion, and partly in response to the effect of cytokines, immunoglobulin type adhesion proteins appear on the endothelial cells.

From the membrane of epithelial cells, the CEA enters the blood stream as well, from where it can be detected. The amount in blood serum is proportional to the progression of the tumor, which makes it ideal for monitoringits development. It may also be useful in detecting relapses after successful treatment. Since other, non-malignant conditions can accompany CEA serum elevation, the laboratory test is not suitable for diagnostic purposes.

The aforementioned adhesion proteins' importance is also underscored by the effect of medicating agents (often prescribed as a preventive measure to at-risk populations –such as patients diagnosed with polyps of the colon) that inhibit the cyclooxygenase-2 enzyme leading to down-regulation of ICAM1 and VCAM1 expression. Aspirin is such an agent for example, as well as the more selective coxibs derived from aspirin.

As with the adhesion of white blood cells, *selectins* in a similar fashion promote the *initial, weak adhesion of tumor cells*, upon which a tighter adherence occurs after integrin activation, followed by diapedesis and exit from the blood stream. E-selectins seem to be important not only in the formation of the initial, transient adhesion, but also appear to contribute to the cells' passage through the endothelial layer (diapedesis), thus its role is multifaceted. Although E-selectins are not present on epithelial cells of the colon, they are greatly expressed in vessels with close proximity to the primary and metastatic tumors, thus it is not surprising, that the soluble E-selectin level in blood correlates with the occurrence of CRC hematogenous metastatic spread. P-selectins bind to the fucosylated, sialyated

surface proteins of tumor cells, making possible the platelet-tumor cell interaction that provides protection from the anti-tumor immune reaction, as well as being important in the intra- and extravasation.

The role of selectins in other aspects of tumor development has been raised. The vascular and endothelial growth factor (VEGF), which tumor cells are capable of producing in large amounts, increases the expression of E-selectins on endothelial cells. Since VEGF is one of the most potent activators of angiogenesis,thereby supplying the tumor with oxygen and nutrients; it is likely that E-selectin also plays a role in this process.There are many hopes connected to the development of inhibitory agents of vessel formation (anti-angiogenic drugs) and their therapeutic potential but in order to experiment with such compounds it is important to identify the possible molecular targets participating in this process which can be acted on by pharmaceutical agents. Wide spread clinical use has been achieved using monoclonal antibodies against VEGF (bevacizumab, Avastin). Blocking type of anti-selectin antibodies, soluble selectin receptors, and selectin antagonists are all in development. Besides experimenting with selectins, inhibitory agents are also being tested on other types of adhesion proteins such as integrins. Promising animal experiments have been performed recently which could prove useful in pharmaceutically blocking the previously mentioned VE-cadherin molecules.

The role of *integrins* in the normal and pathological functioning of cells is extraordinarily diverse. The binding which accompanies integrins is ten times as strong as with selectins, which allows loosely attached tumor cells to achieve a tighter connection with the endothelium (analogous to the mode of leukocyte adhesion – see below). Based on the transduction signals initiated by integrins, endothelium retraction is induced, which in turn facilitates the extravasation of tumor cells (this has less meaning in the sinusoids where the endothel layer is fenestrated). Furthermore, they participate in apoptosis, cell migration, tumor invasion, and angiogenesis. It has been observed how the pattern of integrin expression on tumor cells changes with tumor growth and development. For example, integrin expression increases significantly on CRC tumor cells, what is more, new and previously unseen subunits appear, changing their composition during proliferation. This results in a significantly greater tendency for groups of cells within the tumor to metastasize.

The αV integrins have more recently been the main focus of studies. Experimental blockage of αVβ6-integrins has resulted in the reduction of uPA (urokinase type plasminogen activator), MMP2 (matrix metalloprotease2) and MMP9 protease secretions, thereby limiting initial ECM breakdown which is one of the key steps of tumor invasion. The αVβ3-integrin (vitronectin receptor) also appears to be meaningful in metastatic progression: human breast cancer and melanoma cells are only able to metastasize in case αVβ3-integrins are present on their surface in an active conformation. Furthermore, blockage of αV integrins has significantly reduced the rate of cell proliferation, while in a few experiments even apoptosis has been provoked.

It has long been known, that surgical intervention and removal of the primary tumor accelerates the growth of metastasis. It has also been observed that upon excision of a large intestinal tumor, the adhesion of circulating tumor cells increases in the liver. This latter phenomenon in rats has been proven to be preventable with monoclonal anti-α2-integrin therapy. Several integrins have furthermore pro-angiogenic effects; their antagonists are being investigated as potential therapeutic agents.

Thus therapeutical modification of cell adhesion shows promising signs of becoming a key element in the arsenal of future oncologists.

Leukocyte extravasation, leukocyte adhesion deficiency

In order for white blood cells (WBCs) to fulfill their role in tissue, they must leave the circulation (extravasation). For this they must first of all cross the otherwise relatively tightly joined endothelial layer. This happens in two steps. First, the circulating leukocytes bind to the endothelial cells and through these cells make their way out of the vessel lumen. Extravasation takes place almost exclusively in the post capillary venules.

First leukocytes establish a loose connection with the endothelial cells, slow down and continue to roll along the vessel wall (*"rolling"*). Selectins have a major role in this initial step of adhesion. Most leukocytes express *L-selectins*, while activated endothelial cells express *E- and P-selectins*. The greatest affinity is shown towards P-selectins, but the other two selectins can also bind with *PSGL1* (P-selectin glycoprotein ligand 1) expressed on leukocytes and according to recent data PSGL1 is also present on certain endothelial cells. The resultant connection is not durable, since the cell drifts further on but does allow for the establishment of additional connections with new selectins, and leads to the leukocyte to continue rolling along the surface of endothelial cells. Upon binding of selectins, intracellular signal transduction mechanisms elicit the appearance of additional adhesion molecules (e.g. integrins). Furthermore it can bring already expressed proteins into active, ready-to-bind conformational states on both WBCs and endothelial cells.

Integrins participate in the process of rolling as well as the establishment of the subsequent tight adhesion. The early binding between PSGL1 and *LFA1* integrins for example creates such an intermediary affinity state, which is suitable for a temporary connection by ICAM1 proteins on endothelial cells. A similar integrin conformational change takes place with *VLA4* (*VCAM1* ligand) and the *Mac1* (mainly ICAM ligands), which allows during the emerging transitional affinity state the establishment of transient connections. These transient connections also favor rolling, albeit at an even slower pace (*"slow rolling"*) compared with the selectin dependent form in the beginning.

Thereafter the rolling leukocytes come to a complete halt due to the effect of cytokines (*chemokines*) being released by cells near the inflammatory site, resulting in tight connections with the endothelial cells (*"arrest"),* in which the earlier mentioned integrin-CAM interactions (LFA1-ICAM, VLA4-VCAM1) play the main role. In order for the above scenario to take place, the endothelial cells must produce such CAM proteins, which can serve as ligands for leukocyte integrins (and vice versa, integrins which are capable of binding with leukocyte CAM proteins). These endothelial cells must be further activated based on the effect of inflammatory cytokines. After such an activation not just the expression of adhesion proteins are increased on endothelial cells, but the synthesis of chemokines and lipid forms of chemoattractant substances are increased to attract more and more WBCs to the affected area. The gradient concentrations of these substances direct the WBCs' extravasation and migration towards the site of inflammation (chemotaxis). To ensure a stable integrin-CAM connection both participating cells must be activated, which in the case of endothelial cells is induced primarily by a cytokine signal, while leukocytes receive their activating signal from chemoattractant molecules released – though frequently produced at a distance and only transported to the luminal side – by endothelial cells. The chemokine effect not only stabilizes the affinity conformation of integrins, but influences their surface arrangement: on active leukocytes integrins are often found in groups (clusters). The exact intracellular signaling pathway responsible for activating integrins is not yet fully understood, but phospholipase C induced intracellular increase of [Ca^{2+}], small GTPase proteins and actin-binding proteins (e.g. talin-1) all certainly play a role in this process.

In order for a leukocyte already successfully attached to a blood vessel wall to penetrate through, it must first find the proper place for transendothelial migration. This is called *"crawling",* when the WBC spreads out, stretches and searches by crawling for the most suitable place to permeate the endothelial layer. The shape change and crawling motion is mediated by Mac1-ICAM1 adhesions. When the WBC has found the proper place, it can start the *transmigration*, upon which it crosses three barriers: the endothelial layer, basal membrane and finally the pericyte layer. The cytoplasmic domains of endothelial CAM proteins are in contact with numerous signal mediating molecules, which are in turn capable of additional protein interactions. In case of ligand binding, a protein cascade is activated,

which ultimately leads to the transformation of junctions between adjacent lying endothelial cells. The preexisting intercellular adhesion connections unravel, the proteins preventing transmigration (e.g. VE cadherin) are removed from the affected region, while junctional molecules with leukocyte type ligands migrate to the luminal surface, so that they may help direct the WBCs through the intercellular gaps. Concerning the latter, most of the junctional proteins actively involved in transmigration are members of the immunoglobulin superfamily, e.g. *PECAM1* (platelet-endothel cell adhesion molecule), *JAM-A*, *-B*, *-C* (junctional adhesion molecules) and *ESAM* (endothel-selective adhesion molecules). While the PECAM1 establishes a homotype connection between the leukocyte and endothel cell, the JAM proteins are capable of homotype and integrin binding as well. By forming and unraveling these connections, leukocytes travel between the endothel cells; we call the latter process *paracellular* transmigration. Naturally for all this to occur the leukocyte must change shape (dynamically unleash and retract pseudopods, drag and pull the lagging part of the cell after itself).

An alternative mode for leukocytes to pass through the endothel layer is via a *transcellular route*. Only a small fraction of the circulating leukocytes take advantage of this form of passage and is only available in certain designated areas of the circulation (e.g. central nervous system, or occasionally inflammatory sites). In these instances, transmigration through endothel cells occurs via so called vesiculo-vacuolar organelles, which transport the leukocytes within narrow, intact bound plasma membranes between the lumen and the basal surfaces. The transport process is primarily initiated by the ICAM1 ligand binding, involving the actin- and vimentin- filament system as well as the caveolin proteins. Concurrently, the ICAM1 molecules translocate to the membrane region lining the channels, while the previously mentioned paracellular adhesion molecules (e.g. PECAM1) also participate in this step. An additional factor contributing to transcellular passage is the endothel cell's active flattening (or shrinkage), thereby shortening the distance to be crossed. The WBCs emit membrane bulges into the channels while their further movement does not differ significantly from paracellular transmigration.

Those leukocytes which succeed with transendothelial migration must also cross the basal membrane and the pericytic layer. This last step to leave the vessel is the slowest and can take as long as 15 minutes compared with the few odd minutes required for the previous steps. The precise mechanism is not entirely clear here either, but we are certain that PECAM1 binding induces additional integrin activation and translocation. Moreover such integrins whose main ligands are laminins, are likely to assist in adhesion to the basal membrane. Leukocyte elastase expression also increases in response to the effects of certain stimuli. Being proteolytic in nature, the enzyme helps degrade the ECM components in and around the vessel wall to achieve penetration into the tissue.

The structure of the basal lamina is not uniform. It has been shown that certain regions contain considerably less laminin and collagen IV. Furthermore the size of such regions increases in response to cytokine stimulus. Such areas not only facilitate the passage of cells, but provide for a freer diffusion of chemoattractant substances (which otherwise bind in large quantities to the heparin-sulfate components of the basal lamina) meaning they may have an important role in navigating leukocytes. These thinner and more permeable parts of the basal lamina happen to fall in the same place as the gaps between pericytes, as well as parts of the vessel wall which contain less ECM proteins and are less dense.

Leukocyte adhesion deficiency

A case study:

"The parents of a 3 year old active, healthy looking and normally developed girl took their child to an oral surgical specialist. Their main complaint was that her gums had been red for three months, were tender, and were accompanied by frequent oral mucosal bleeding. Her parents added that their daughter for the past year had suffered reoccurring symptoms of epidermal inflammation with associated skin peeling as well as reoccurring and difficult to treat urinary tract infections. Upon

medical inspection of the daughter's oral cavity a diffuse, blood rich, swollen buccal mucosal surface was found, which started bleeding after mild pressure was applied. Inside the mouth on the surface of the lower lip an approximately 1 cm diameter ulcer was visible, which was missing the usual fibrin-purulent membrane coat from its surface. An enlarged lymph node was not palpable and the result of a head-neck radiological examination was also negative. A microbiological test of the mucous isolated from the periodontal region confirmed only the presence of bacteria and fungi belonging to the normal flora of the oral cavity. Although these species rarely cause disease, the girl was prescribed targeted antibiotics and antifungal therapy for 3 weeks. Despite the therapy her symptoms did not improve substantially requiring the girl to be referred to the immunology wing of the pediatric department. Subsequent laboratory tests indicated a WBC count above 30000/µl (normal range: 5000-13500/µl), of which neutrophil granulocytes represented 24600/µl of the absolute total amount (normally neturophils compose 60-70% of WBCs). Flowcytometry studies revealed a reduced expression of surface proteins CD11a, CD11b, CD11c and CD18 on granulocytes and lymphocytes. Additional laboratory tests signaled significantly reduced chemotactic and bactericidal function of WBCs. While performing a cytogenetic analysis, 2 point mutations were discovered on the q arm of chromosome 21. Based on the evidence at hand type I leukocyte adhesion deficiency was confirmed. Months after establishing the diagnosis, a severe epidermal inflammatory reaction occurred on the girl's arm after being bruised, which failed to heal and finally required an autologous skin graft transplantation. Three months later the girl suffered a serious intestinal infection with accompanying diarrhea, which could only be managed with a surgical procedure called a colostomy (an artificial opening in the abdominal wall allowing feces to pass into a bag). Within a short period of time a strain of bacteria colonized the area surrounding the large-intestinal stoma which exhibited a high degree of resistance to antibiotics. The patient received granulocyte concentrated transfusions for 2 weeks, followed by an allogeneic (donor derived) bone marrow transplantation which was rejected however. Two months after the transplantation a severe febrile infection developed, this was followed by acute hypotension and renal failure. Ultimately respiratory insufficiency ensued, which is why the girl was transferred to the pediatric intensive care unit and was placed on an artificial ventilator. After exhausting all other possible treatments and taking into consideration the poor prognosis, the parents and their doctor made the joint decision a few days later to cease artificial ventilation, leading to the girls passing (exitus letalis) 26 months after the diagnosis had been made for LAD 1."

Leukocyte adhesion deficiency (LAD) is an inherited disease, which is characterized by recurring skin- and mucosal infections, subcutaneous infections, reduced purulence (pus) formation and poor wound healing. The latter includes from experience a warning sign among newborns related to the umbilical stump failing to dry and detach properly. Besides this, such infants are more susceptible for all kinds of infections (mainly of bacterial and fungal origin) than the general population. The course of the resultant infection is more severe, and the new born child's prognosis is poorer. Routine laboratory studies show a high WBC count with a predominance of polymorphonuclear (PMN) cells. The illness does not have a special therapeutic course, although infections are treated with antibiotics and preventative measures emphasize maintaining a high degree of oral and skin hygiene.

The disorder is subdivided into the following types:

In *type I (LAD I)* a genetic defect impairs the function of adhesion type glycoproteins normally found on granulocytes, monocytes and lymphocytes. Three types of integrin molecules are concerned: *Mac1, LFA1* and *p150,95*. Their β2-subunits are identical, also known as CD18 and their glycoprotein structure differ only in respect to the α-subunit, which are named respectively CD11a, CD11b, CD11c. The Mac1 is found on cells capable of phagocytosis, partially as a complement receptor and partially to function as an adhesion molecule. LFA1 is found on lymphocytes, and are responsible for the cytotoxic

activation of T-cells as well as interactions with other lymphocytes. P150,95 is present on phagocytic cells and lymphocytes however its exact physiological significance is still unclear. The *CD18 gene* is found on region q22.3 of chromosome 21 and a *mutation* concerning this gene locus is seen in LAD 1 patients who undergo genetic testing.

LAD type II is caused by *missingcarbohydrate chains* normally serving as E- and P-selectin ligands (a tetrasaccharide called sialyl-Lewis^X antigen). Being much rarer than type I, only a few cases have been documented and most of these concerned children born to parents who were related. The oligosaccharide chain contains sialic acid and fucose, the latter being incorporated into proteins in GDP fucose form within the glicosylating Golgi apparatus. The underlying cause for this particular subset of disease is the enzyme responsible for GDP-fucose transport, namely *fucosyltransferase*. Although defective fucosylation and the resultant error prone function of selectins is clearly a cause of adhesion problems, at the same time such patients also experience certain kinds of developmental issues (psychomotor and morphological) with a yet to be determined pathophysiological course.

Recently a type III form of the disease was also elucidated, which differs from type I in that the expression of integrins and their structure are normal however their *in situ* chemokine dependent activation is defective. Hence WBCs are capable of "rolling", but the active conformation needed for high affinity integrins to "arrest" and accomplish transendothelial migration fails to occur. A genetic defect associated with a G-protein receptor (GPCR) impairs the proper transduction of arriving signals, which in turn affects the related down-stream protein cascade. The symptoms are similar to those experienced in other forms of the disease, however in LAD III haemophilia is also present, which can be explained by the fact that the GpIIb/IIIa integrin's activation by thrombin, ADP and adrenaline(which is a key step of thrombocyte aggregation) is similar to the chemokine dependent activation of the aforementioned integrins.

Questions:

1. How do you interpret the relationship between genetic defects and the resulting clinical manifestations thereof? Which steps of the immune response are impaired?
2. How would you explain the failure of the umbilical stump to dry and detach properly as one of the frequent signs of the disease? What role could granulocytes possibly have in this regard?
3. *In vitro* tests performed on leukocytes obtained from LAD I patients not only show adhesion but phagocytic impairment as well. What could be the cause of this?
4. In case we only refer to the CD18 gene defect, why does cell-surface expression of α-subunits also decrease?
5. The life-span of WBCs provided in a dose of granulocyte concentrate is only a few days. Furthermore, these foreign WBCs provoke an immune reaction and potentially weaken an already compromised immune system. Despite all this what could be the reasoning behind administering such a preparation?
6. What did the doctors treating their patient hope to achieve with an allogenous bone-marrow transplantation?
7. Which treatment options still at their experimental phase would you consider as a possible cure for this illness in the future?
8. In a related case, similar clinical manifestations can frequently occur when there is a NADPH-oxidase error causing a *chronic granulomatous disease* or when someone suffers from *Chediak-Higashi syndrome* associated with a defect in lysosomal breakdown capacity. Which laboratory methods would you use in order to make a differential diagnosis with respect to LAD?

Recommended literature

1. A.Etzioni, Defects in the Leukocyte Adhesion Cascade. *Clin Rev Allergy Immunol* 2009 May 13 E-pub ahead of print.

2. V. Bogomolski-Yahalom, Y. Matzner, Disorders of neutrophil function. *Blood Rev* 1995 Sep;9(3):183-90.

3. K. Ley, C. Laudanna, M.L. Cybulsky and S. Nourshagh, Getting to the site of inflammation: the leukocyte adhesion cascade updates, *Nat Rev Immunol* 2007 Sep;7(9):678-89

4. P. Gassmann, A. Enns and J. Haier, Role of tumor cell adhesion and migration in organ-specific metastasis formation. *Onkologie* 2004 Dec;27(6):577-82.

5. C. Kneuer, C. Ehrhardt, M.W. Radomski and U. Bakowsky, Selectins – potential pharmacological targets? *Drug Discov Today* 2006 Nov;11(21-22):1034-40.

6. R.K. Andrews and M.C. Berndt, Platelet physiology and thrombosis. *Thromb Res* 2004;114(5-6):447-53.

7. R. O. Hynes, Integrins: Bidirectional, allosteric signaling machines. *Cell* 2002;110: 673-87.