Cell adhesion molecules

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Introduction

One of the areas of biomedical science that has advanced tremendously in the past decade is that of the understanding of signalling at the cell surface. It is becoming increasingly clear that the body's tissues rely, in the main, for their integrity, function and spatial organisation on interactions at the cell surface between cells and their soluble and insoluble molecular environment. The molecules that facilitate interactions between cells and between cells and tissue matrices are known as cell adhesion molecules.

Types of cell adhesion molecules

There are six known cell adhesion molecule families into which most of these entities are grouped on the basis of chemical, structural or functional similarities. Four rely on interactions between proteins and two on interactions between proteins and carbohydrates. The four protein–protein recognition molecules are: (a) the immunoglobulin superfamily; (b) the cadherins; (c) the integrins; and (d) the receptor protein tyrosine phosphatases. The selectins and hyaluronate receptors mediate interactions between proteins and carbohydrates. All but the hyaluronate receptors are involved in cell–cell adhesion; integrins and hyaluronate receptors are involved in cell–matrix interactions.

All of these molecules are transmembrane glycoproteins with extracellular binding domains and cytoplasmic functional domains. On binding of ligands to the extracellular domain intracellular events are initiated through the cytoplasmic functional domain. In turn, these cause major behavioural and functional changes in the cells. Thus if cells are to interact with one another or with their matrix, two complementary molecules are required, the adhesion molecule and its ligand, one on each side of the adhesion site. These may be: (a) members of the same group of cell adhesion molecules (homophilic)—for example, cadherin–cadherin binding; (b) members of different groups of cell adhesion molecules—for example, selectins binding to members of the immunoglobulin superfamily; and (c) a cell adhesion molecule and a molecule which does not belong to any of these groups—for example, integrins binding to matrix molecules.

ADHESION MOLECULE GROUPS

The immunoglobulin-like superfamily

This is a large and diverse family of molecules so named because they have one or more immunoglobulin-like domains.¹ Included within the group are molecules which mediate antigen recognition by, and adhesion to,

lymphocytes—for example, CD3, CD4 and CD8, which together recognise complexes of antigen peptide and the major histocompatibility complex on other cells, and lymphocyte function associated (LFA) antigens such as CD2.

Other important molecular subgroups in this superfamily are the intercellular adhesion molecules (ICAMs), which are widely expressed on epithelial and endothelial cells; nervous system adhesion molecules—for example, neural cell adhesion molecules (N-CAM); and molecules such as L1 and TAG which are involved in the organisation and function of nerves.

Cadherins

The calcium dependent cell adhesion molecules (cadherins) are so called because they have both adhesion and calcium binding sites.² Classically, these molecules were thought to be homophilic. Intracellularly, they attach to a group of molecules known as catenins which link the cytoplasmic domain of the cadherin molecule to intermediate filaments of the cytoskeleton.³

Of the molecules in this group, the best characterised is E-cadherin, the expression of which is regulated by the ErbB2 proto-oncogene.⁴ It is necessary for early organisation of the developing embryo and thus is one of the first adhesion molecules to be expressed during embryonic development. E-cadherin is concentrated in an intercellular junction known as the zonula adherens⁵ and is attached to the intermediate filament actin.

Cadherins are also important components of desmosomes—for example, desmoglein and desmocollin,⁶ which attach to cytokeratins.⁷

Recent studies of cadherins have revealed that these molecules are more diverse than originally thought. Once believed to be homophilic molecules deployed in epithelial type intercellular binding, there is now evidence that binding between T lymphocytes and epithelial cells can be mediated through E-cadherin binding to α E/ β 7 integrin,⁸ a form of heterophilic binding. A new group of cadherins (type II) that mediate mesenchymal loose adhesion has been described recently. One in particular, cadherin-11 (cad-11, formally known as OB-cadherin and first identified on osteoblasts⁹), is expressed widely during embryogenesis and may be a pivotal molecule in mesenchymal organisation.¹⁰

Integrins

The integrins are both cell–cell and cell–matrix adhesion molecules.¹¹ They are heterodimers consisting of one α and one β chain, both of which are necessary for binding. Adhesion also requires the presence of divalent cations such

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as Ca^{2+} and Mg^{2+} . To date, 14 α and eight β chains have been described. Theoretically, any combination of α and β chains could exist, but only limited permutations have been identified so far. Integrins are subclassified according to which β subunit is involved in the complex.¹² There are three main classes of integrins: $\beta 1$, $\beta 2$ and $\beta 3$. The $\beta 1$ and $\beta 3$ subfamilies predominantly mediate cell–matrix interactions, while the members of the $\beta 2$ class are cell–cell adhesion molecules. There is an additional distinction between the $\beta 1$ and $\beta 3$ integrins in that the $\beta 1$ integrins are generally involved in adhesion to connective tissue macromolecules, such as fibronectin, laminin and collagens, whilst the $\beta 3$ integrins bind to vascular ligands, such as fibrinogen, von Willebrand factor, thrombospondin, and vitronectin. In terms of cellular distribution, $\beta 1$ and $\beta 3$ integrins are coexpressed on most cell types, whereas $\beta 2$ integrins are restricted to leucocytes.

Some of the integrins are highly specific in their ligand binding properties, recognising short amino acid sequences on the cell surface and matrix proteins. Others are less specific. Thus, $\alpha 5 \beta 1$ binds to the tetrapeptide arginine-glycine-aspartate-serine (RGDS) on fibronectin, whereas $\alpha 2 \beta 1$ binds to amino acid sequences on collagen, fibronectin and laminin.

Selectins

Most cellular adhesion interactions require protein–protein binding. Selectins, however, have lectin-like (carbohydrate binding) domains on the extracellular component of the molecule.¹³ There are three major groups of selectins: the L-selectins (the main example of which was once known as LCAM or MEL,¹⁴ which are homing receptors for specific adhesion of lymphocytes to endothelial cells of peripheral lymph nodes; the E-selectins (endothelial leucocyte adhesion molecules (ELAMs)), which are important mediators of inflammatory reactions and are upregulated within hours by inflammatory mediators; and P-selectin, which is contained in Weibel-Palade bodies of endothelial cells and α granules of platelets. This is released during clotting and at times of platelet activation, and mediates adhesion between leucocytes and platelets.

Hyaluronate receptors

The fifth group of adhesion molecules is defined functionally, rather than structurally. Interest in this group has increased recently because its members play a key role in determining growth, differentiation and tumour progression.

Hyaluronate is an abundant saccharide component of extracellular matrices that is believed to be important in a variety of pathological processes, including inflammation and tumorigenesis. Hyaluronate is hydrophilic and, in association with other matrix molecules, has the capacity to take on a macrostructural role. This generally occurs in relatively hypocoelular gels, such as cartilage and

synovial fluid. In addition, hyaluronate has numerous other functions, including promotion of cell growth and migration during embryogenesis and in later life.

Several proteins have specific affinity for hyaluronate, including the matrix proteoglycans link protein and aggrecan. Cells bind to hyaluronate through cell surface receptor proteins. Only two have been characterised to date: CD44¹⁴ and receptor for hyaluronate mediated motility (RHAMM).¹⁵ RHAMM is upregulated in H-*ras* transformed cells that exhibit increased motility, which can also be induced in cells expressing RHAMM either by contact with antibodies directed against RHAMM or with hyaluronate. RHAMM has been identified on the surface of migratory activated T lymphocytes, but not on those that are relatively fixed.¹⁶ Similar findings have been reported in activated B lymphocytes.¹⁷

CD44 displays heterogeneity through alternative splicing of its exons, resulting in various isoforms which can be non-hyaluronate binding, hyaluronate binding when activated (for example, if stimulated by phorbol ester), or constitutively binding.¹⁸ The CD44 isoforms have a variety of physiological roles, including lymphocyte homing, immune response regulation (through lymphocyte activation) and cell migration.¹⁹

Receptor protein tyrosine phosphatases

The receptor protein tyrosine phosphatases (RPTPs) are involved in intercellular signalling and regulation of cell–cell adhesion.^{20–21} The intracellular domains of other adhesion molecules bear no resemblance to known signalling molecules. To initiate a signalling response they must work indirectly through associated molecules. In contrast, the RPTPs have catalytically active, cytoplasmic tyrosine phosphatase domains and therefore have the potential to modulate directly catalytic events involved in cell signalling. Their extracellular domains are diverse but many resemble cell adhesion molecules. PTP μ and PTPk²² mediate homophilic cell aggregation and a soluble fragment of PTPzeta/ β (phosphacan) binds to Ng-CAM, N-CAM and tenascin.²³ Some RPTPs have been localised to points of cell–matrix contact, although a role in cell–matrix binding has yet to be established fully.²⁴ The physiology of this group of molecules is largely unknown, but they are undoubtedly fascinating and will stimulate interest for years to come.

Function and actions of cell adhesion molecules

Some of the functions of cell adhesion molecules have been alluded to earlier. It is apparent that, at a cellular level, cell adhesion molecules are responsible for more than just adhesion of cells to one another and to their insoluble matrices. Additional functions include: the ordering of cell sorting, migration and differentiation; organisation of cell motility via the cytoskeleton; regulation of inter- and intracellular signalling; and control of gene transcription. In recent years, there has been an

Figure 1 E-selectin expression in a T cell lymphoma infiltrating subcutaneous fat. (IHC; original magnification $\times 50$.)

Figure 2 $\beta 1$ integrin expression in chondrocytes in human fracture callus. (IHC; original magnification $\times 50$.)

explosion of interest in the function of these molecules in all of these areas and it is impossible to be exhaustive here. In the rest of this review specific areas have been selected to highlight the changing perspectives of the function of cell adhesion molecules.

Cell-cell adhesion

Cell to cell adhesion is obviously one of the most important functions of cell adhesion molecules. Adhesion of one cell to another serves two major purposes. The first is to form distinct groups, lines or sheets of cells and the second to prevent or arrest movement.

One of the most striking electron microscopic features of epithelial and non-epithelial tissues alike is the presence between cells of junctional complexes specifically designed to lock cells to one another. The most dramatic of these structures are the desmosomes. As stated earlier, cadherins are important structural and functional components of desmosomes. Perhaps the most recent notable advance has been the determination of two high resolution structures on the extracellular cadherin domains implicated in cadherin-cadherin binding.²⁵ It has been suggested that the two molecules may

form parallel dimers leading to a zip-like structure as the basis of adhesion. This analogy may give an important insight into the function of cell adhesion molecules during cell migration where the rapid attachment and release of cell processes from other cells and their matrices are implicit in directed cell movement.²⁶ Other theories suggest that the cadherins do not function in this way but form rod or cylindrical oligomers bridging the space between cells.²⁷ The other recent advance which has already been alluded to also refers to cadherins. These are associated traditionally with extensive interactions between cells. It is recognised now that there are also weaker binding cadherins that allow looser clusters of cells to form.²⁸ The mechanism underlying this is not known but they still link to intracellular catenin polypeptides.

There has also been an understandable upsurge of interest in disturbed cell-cell adhesion as part of pathological processes. The possible importance of E-selectin in the dermatotropism of cutaneous T cell lymphomas is inferred by the expression of the E-selectin ligand, CLA, on the malignant cells in most cases of mycosis fungoides (fig 1).²⁹ There is also evidence that the expression of carbohydrate determinants for E-selectin binding is related to the metastatic potential of colon carcinoma cells.³⁰ By a similar mechanism, P-selectin on endothelial cells could also be involved in the metastatic arrest of tumour cells bearing appropriate carbohydrate epitopes. The interaction of activated platelets with tumour cells via P-selectin may facilitate metastasis by the physical arrest of the tumour cell-platelet aggregates in the microvasculature or by the provision of platelet growth factors to the co-aggregated tumour cells.³¹

Cell-matrix adhesion

Integrins are the predominant mediators of cell-matrix adhesion (fig 2). It is now clear that these molecules are a key part of a very complex organisational hierarchy that controls cell function and movement over matrices. In monolayer culture, integrins cluster within focal adhesion complexes on regions of close contact between the cell and its matrix. There is evidence that these clusters induce changes within the cell, leading to recruitment of a number of structural proteins to the developing adhesion complex and generating a physical link with the actin based cytoskeleton.³² The structure of the complex is independent of the nature of the integrin or the cell. There is also built in molecular redundancy within the complex; thus, genetic ablation of the ubiquitous actin binding protein vinculin does not inhibit greatly the formation of adhesion complexes or links between the complex and the actin cytoskeleton.³³ However, factors that influence the cell profoundly are the chemical and physical nature of the matrix to which they are attached.³⁴ These can affect cell shape and, more importantly, cell proliferation and differentiation.³⁵

Regulation of cell signalling

In addition to their role in cell–cell adhesion, cell adhesion molecules are involved in the transmission of signals across cell membranes. Signals can be of two types. The first and more obvious is signal passage from the outside of the cell to the inside in response to ligand binding, with resulting activation of second messenger systems and gene transcription. Such signals are responsible for functions as diverse as stimulation of anchorage dependent cell growth, differentiation and protection from apoptosis.^{36–37} The second signal type is passage of information from the inside of the cell to the outside (inside out signalling). This usually modulates the binding affinities of cell adhesion molecules.³⁸ It is implicit that for cell adhesion molecules to be involved in both mechanisms, both inter- and intracellular signalling pathways must be focused upon cell adhesion molecules or other molecules concentrated in their immediate vicinity.

Although not strictly cell adhesion molecules, the connexins are a family of (currently) 12 transmembrane proteins that form hemichannels on each of two opposing cells. When the cells come into contact a complete intercellular channel (a gap junction) is formed. These channels provide a direct pathway for the passage of small molecules (1–2000 daltons) between cells.³⁹ The connexin family seems to have an important role in morphogenesis and the function of excitable tissues. The importance of this role is exemplified by Charcot-Marie-Tooth disease which has been shown to be causally linked to a genetic mutation in connexin 32.⁴⁰

Intercellular signalling pathways mediated through other specialised cell junctions have also been the subject of recent intensive investigation. In lower animals a group of proteins known as the membrane associated guanylate kinases (MAGUK) has been identified.⁴¹ These molecules are important structural components of tight junctions and synapses, and also participate in cell signalling pathways.⁴² Their concentration at tight junctions indicates an association with cell adhesion molecules,⁴³ but whether there is a functional relation other than their using the coincidental proximity of the two cells at these sites remains to be established.

In terms of true cell adhesion molecules, there has been a particular interest in intracellular signalling pathways initiated by ligand binding to integrin molecules. Binding of integrins to ligands induces phosphorylation of protein kinases. It is now firmly established that a major substrate for integrin induced tyrosine phosphorylation is the protein tyrosine kinase focal adhesion kinase (FAK), which initiates a very sophisticated intracellular signalling pathway linked to the integrin receptor.^{44–45} There are, however, many other signalling mechanisms that can be activated by integrins. These include Ca^{2+} flux, intracellular alkalisation, accumulation of signal transduction adaptors and enzymes (for example, phosphoinositides and protein kinase C), arachidonic acid pathways, activation of cyclins and the

retinoblastoma gene, and activation and inhibition of regulators of apoptosis.⁴⁶ Allied to this, a large number of cytoskeletal and signal transduction molecules are bound to, or spatially modulated by, integrins, including talin, α -actinin, tensin, vinculin, actin, paxillin and filamin, Src family kinases (for example, c-Src, c-Fyn), Src substrates (for example, FAK, pp120, cortactin), growth factors and their receptors (for example, FGF receptor, heparin binding EGF-like growth factor), and other transduction factors (for example, c-CSK, PLCgamma, Rho, Grb2, Ras, MEK, and ERK).

Other cell adhesion molecules have also been studied but their pathways are less well described than those of the integrins. Recently, for instance, it has been shown that cadherins, once believed to play roles only in cell adhesion, sorting and migration, are also responsible for cell signalling. β -catenin, for example, is an essential component of the Wnt signalling pathway that mediates developmental patterning in lower animal embryos.⁴⁷

It must be stressed that there is no evidence to suggest that the intracellular signalling pathways activated by binding of cell adhesion molecules to their ligands are in any way specific, but are the same second messenger systems that are activated by many other molecule receptor/ligand binding systems.⁴⁸

Cell motility via the cytoskeleton

Cell adhesion molecules are believed to play an important role in controlling cell migration through their connections with the cytoskeleton—for instance, the cadherins which are concentrated in the zonula adherens.⁵ Through their cytoplasmic domains they associate with catenins, which in turn are attached to intermediate filaments of the cytoskeleton,³ notably the contractile molecule actin. Similarly, molecules such as talin and α -actinin link the cytoplasmic domain of β 1 integrins to actin filaments.⁴⁹ It is also known that in certain disorders where cell adhesion molecules are abnormal, such as leucocyte adhesion deficiency, which is characterised by the absence of the β 2 subunit of the integrin receptor, the function of the cell, in this case adhesion of a leucocyte to endothelium, is abnormal.

Despite observations such as these, the role of cell adhesion molecules and their receptors in the control of cell movement via connections with the cytoskeleton are still poorly understood. A key problem has been a limited understanding of the dynamics of the cell membrane. Recent biophysical studies have shown that these structures are much more complex than had been previously recognised.^{50–51} One exciting outcome has been the observation that the transmembrane proteoglycans, like the cell adhesion molecules, may undergo directed movement within the membrane, for instance towards the leading edge (as opposed to the trailing edge) of the cell. Conversely, they may remain stationary whilst other molecules move about them. Apparent deviations from the expected Brownian motion

Figure 3 Upregulated type I collagen gene expression in osteoblast-like cells after microloading on a mesh (deformable matrix). (NISH, original magnification $\times 200$.)

of these molecules may play a significant role in the function of cell adhesion molecules. This process of confined diffusion has been reported for a number of cell adhesion molecules including E-cadherin⁵² and N-CAM. However, the study of the plasma membrane dynamics of cell adhesion molecules, although having enormous implications for the understanding of the functions of cell adhesion molecules, has only just begun.

Cell motility probably involves the regulation of cell adhesion via a cycle of attachment and detachment. A cell extends a process that becomes tightly adherent to the matrix or another cell. Force is generated to pull the cell forward, whilst detachment occurs at the trailing edge. Adhesion in motility is therefore controlled both spatially and temporally.⁵³ Integrins are important in cell migration over matrices. For example, the integrins $\alpha 5 \beta 1$ and $\alpha 4 \beta 1$ confer migration on fibronectin⁵⁴ and $\alpha 2 \beta 1$ migration on collagen.⁵⁵ Furthermore, correlations have been described between the integrin repertoire and tumour behaviour—for example, expression of $\alpha v \beta 3$ correlates with melanoma invasion.⁵⁶ Our understanding of the way that cells function indicates that this must be regulated both by external influences via signals from cell surface receptors and by intrinsic cellular factors. In this respect, interest has been increased by the observation that focal adhesions and actin membrane interactions might be regulated by signal transducing molecules, associated with the cell adhesion molecules, such as the rho family of GTP binding proteins and by tyrosine phosphorylation of focal adhesion proteins.⁵⁷ There is also increasing evidence that there is significant cross-talk between adhesion molecule modulated and non-adhesion molecule modulated migration promoting factors, such as certain growth factors (for example, EGF) and cytokines (for example, interleukin-8), signalling pathways regulating protein activation, such as phosphorylation of the EGF receptor, and genes controlling secretion (for example, matrix degrading enzymes including the MMPs). This results in highly interactive and complex

regulation of migration and, through inside out signalling, cell adhesion molecule expression.

Regulation of gene transcription

As has been discussed already, cell adhesion molecules, via their intracytoplasmic domains, can both influence cell shape through their attachments to elements of the cytoskeleton and stimulate common second messenger systems. There is evidence (direct and indirect) that, through these connections, cell adhesion molecules can also influence gene transcription. This is best exemplified by examining the effects of alterations in matrix on gene expression by cultured cells. In such experiments the general culture conditions are kept uniform and the only factors that change are the shape or structure, or both, of the matrix.⁵⁸

The method by which these interactions affect gene transcription are only partially known and their study has been based on the logical dissection of transcription pathways. One particular area of study has centred on transcription factor cascades. Experiments on hepatocytes in culture have shown that if they are maintained on a malleable matrix, expression of the immediate early response genes *jun*, *fos* and *myc* is downregulated (relative to gene expression on cells maintained on rigid matrices), differentiation of the cell occurs and the DNA binding activity of transcription factors such as AP-1 is attenuated (fig 3).⁵⁹ In addition expression of the liver specific transcription factors HNF3 α and eH-TF, which modulate the albumin gene enhancer, is upregulated in a cell shape dependent manner.⁶⁰

It has yet to be elucidated fully whether these effects on gene transcription are mediated via second messenger systems or the cytoskeleton. It is of interest to note, in this context, that the nuclear skeleton has attachments to the cytoskeleton⁶¹ through nuclear laminins,⁶² and the nuclear skeleton incorporates a series of protein fibres that bind to DNA at matrix attachment regions. It is possible that cell adhesion molecules cause changes in the cytoskeleton which in turn restructure the nuclear matrix, initiate changes in DNA binding and thereby influence gene expression. For example, in differentiating osteoblasts cell adhesion molecule mediated transfer from a proliferative to a secretory phase results in profound changes in the composition of the nuclear matrix with the associated production of a novel nuclear protein NMP-2, which binds to the promoter of the osteocalcin gene and induces its expression.⁶³

Cell migration, sorting and differentiation

Nowhere is the interplay between cell migration, sorting and differentiation better illustrated than in the area of embryogenesis and morphoregulation. Indeed, one of the major factors initiating the intense examination of cell adhesion molecules was the study of the cell sorting phenomena thought to orchestrate morphogenesis. The organisation of multicellular organisms requires the selective associ-

Figure 4 Complex interactions of cadherin and catenin signalling pathways that may influence embryogenesis. β -catenin links cadherins to α -catenin which in turn connects to actin. Ligand binding to cadherin can result in changes in cell motility and gene transcription through this route. The EGF receptor is preferentially expressed on cell membranes in the vicinity of the cadherin/catenin complexes. It too binds to β -catenin. The β -catenin homologue plakoglobin is a tyrosine phosphatase substrate and can participate directly or indirectly in signalling pathways. Cytosolic β -catenin can also bind to other molecules such as the tumour suppressor gene product APC.

ation of embryonic cells into specific tissues. The pattern of expression of cell adhesion molecules, notably the members of the N-CAM and cadherin families, indicates that these molecules play a pivotal role in linking the primary processes of cell division, migration, differentiation, and cell death. Their spatiotemporal expression suggests that these molecules are key candidates for controlling morphoregulation.⁶⁴ More is known, albeit often indirectly, about the role of the different families of cell adhesion molecules in this context than any other.

THE IMMUNOGLOBULIN SUPERFAMILY

Interference with N-CAM molecules and their expression together with gene knockout experiments have shown that CAMs are important regulators of tissue morphology. Genetic manipulation of mice leading to a failure to express N-CAM causes distortion of the central nervous system. Humans with mutations in the gene encoding L1 (a member of the Ng-CAM subfamily) have a variety of syndromes, including X-linked hydrocephalus, X-linked spastic paraplegia and MASA syndrome.⁶⁵ The molecular events underlying these syndromes are beginning to come to light with evidence that cell adhesion molecule expression is mediated by the morphoregulatory genes of the homeobox family.⁶⁶ Furthermore, the cell adhesion molecules modulate their effects through a whole variety of second messenger systems including those dependent upon inositol phosphate turnover, G protein and changes in intracellular pH.⁶⁷

CADHERINS

The segregation and remodelling of embryonic tissues is associated with sequential expression of different cadherin molecules (fig 4). Cadherins are one of the first type adhesion molecules to be expressed during embryonic development and are necessary for early organisation of the developing embryo. For example, ectoderm surrounding the embryo initially expresses E-cadherin. Yet, when the dorsal ectoderm is induced to form neural tissue by underlying mesoderm, E-cadherin expression disappears, coinciding with the onset of N-cadherin expression in the neural plate. Blocking maternal cadherin by the injection of antisense oligonucleotides results in decreased cell adhesion in the blastomere and disruption of the blastocoel.⁶⁸ A recent study in which antisense oligonucleotides for β -catenin mRNA were injected into developing *Xenopus* embryos lead to the duplication of the dorsal body axis.⁶⁹

N- and R-cadherins are important in the sorting of functional nerve fibre tracks.⁷⁰ Similarly, N-, E- and T-cadherin delineate different regions in the developing spinal cord. The complementary distribution of these molecules is particularly evident in dorsal root ganglia. The spatiotemporal pattern of cadherin expression is consistent with a role for T-cadherin in contact mediated axon guidance in the ventral spinal cord.

HYALURONATE RECEPTORS

Much is known of the distribution and amount of hyaluronates in the developing embryo.

Figure 5 Upregulated $\beta 2$ integrin expression on transmigrating leucocytes. (ISH; original magnification $\times 150$.)

However, although it is assumed that hyaluronate receptors must be important in embryogenesis, little is known of their role. CD44 is found in that portion of the somite where neural crest cells are found during their migration.¹⁹ During heart development, CD44 is expressed in every chamber, which corresponds to the distribution of hyaluronate, the most abundant of the early extracellular matrix components of the developing heart. As might be predicted from its distribution in later life, hyaluronate is found throughout limb buds. CD44 is expressed in the limb bud early in development and antibodies directed against CD44 epitopes block chondrogenesis in cultured limb bud mesodermal cells.⁷¹

Regulation of cell adhesion molecules

As with many other molecules, the regulation of cell adhesion molecules, whether it be stimulatory or inhibitory, may be directed at the molecule itself or at its functional pathways. It is perhaps best to consider regulation of cell adhesion molecules as occurring in three different areas—at the level of the cell adhesion molecule; through the second messenger or cytoskeleton; or at the level of the gene.

REGULATION OF THE LEVEL OF THE CELL ADHESION MOLECULE

The expression of cell adhesion molecules may be upregulated through a number of different mechanisms. The presence of other cell adhesion molecules will induce altered cell adhesion molecule expression or alterations in the distribution of these molecules on the plasma membrane. Such molecules may be on other cells or in the matrix.

The influence of cell adhesion molecule expression on cell trafficking from blood to sites of inflammation has been widely studied. For instance, the endothelium becomes more adhesive for circulating inflammatory cells following altered, often sequential, expression of different families of cell adhesion molecules⁷² as the inflammatory process progresses. Interestingly, the transient presence of leucocytes on the endothelium also stimulates expression and

activation of cell adhesion molecules on the leucocytes themselves.⁷³ Thus, there is evidence that the presence of two cells in contact with one another can lead to upregulation of mutual cell adhesion molecules on both cells. Specifically, in leucocyte trafficking the $\beta 2$ integrins on the leucocytes (fig 5) interact with members of the immunoglobulin superfamily expressed by endothelial cells, resulting in stronger adhesion and leucocyte spreading.⁷⁴

Matrix molecules may have very similar effects. For instance, hyaluronates are major components of early granulation tissue. Investigation of the processes of wound healing by examining the expression of the hyaluronate receptors CD44 and RHAMM has shown that mesenchymal cells in fresh wounds express greater numbers of CD44 transcripts than in normal skin and that this process is associated with a greater collagen synthesis. CD44 is not expressed in fetal wounds and these wounds do not heal with scarring. Thus, the presence of hyaluronates in the extracellular matrix causes upregulation of an adhesion molecule on cells that constitutively expresses this molecule and by this mechanism lead to a change in the function of the cell.⁷⁵

Antibodies can bind to cell adhesion molecules. Depending upon their exact conformation relative to the cell adhesion molecules, they may either inhibit or, by acting as a ligand, activate the processes normally mediated by the cell adhesion molecule. For example, when antibodies directed against CD44v are applied to T lymphocytes *in vitro*, they inhibit the development of an adequate immune response, demonstrating that changed CD44 status determines immune competence rather than being a reaction to it.⁷⁶ A naturally occurring antibody directed against a cell adhesion molecule is found in pemphigus. Here, the characteristic blistering is caused by antibodies directed against desmosomal constituents, particularly the cadherins.⁷⁷ The mechanism is not, unfortunately, mediated by a change in function of the cell adhesion molecule, but by complement induced antibody mediated damage which causes the desmosomes to break down with separation of keratinocytes and intra-epidermal blister formation.

Cell adhesion molecules sometimes interact with one another to modulate their effects. The receptor protein tyrosine phosphatases have some interesting functions that mediate the activity of other cell adhesion molecules. PTP μ will form stoichiometric complexes with cadherins and catenins, suggesting that it is important for the modulation of cadherin mediated cell adhesion. Similarly, the $\alpha 5 \beta 1$ integrin receptor regulates the ability of $\alpha v \beta 3$ to mediate cell migration on vitronectin.⁷⁸ The exact nature of this mechanism is not known.

Matrix binding cell adhesion molecules can also be inhibited. For instance, the $\alpha 5 \beta 1$ integrin binds to RGDS on fibronectin. Binding of integrins to short ligands such as RGDS can be inhibited by synthetic molecules containing the tripeptide RGD.⁷⁹ A recently discovered, naturally occurring family of short integrin ligands (the disintegrins), also con-

Figure 6 *Hox-B8* gene expression in human Pagetic osteoblasts. (ISH; original magnification $\times 100$.)

taining RGD sequences, inhibit normal integrin–ligand binding.⁸⁰ They are more potent inhibitors of $\beta 1$ and $\beta 3$ integrins than synthetic peptides. An example, and one of the first to be recognised, is a disintegrin in viper venom that is an inhibitor of platelet aggregation mediated by $\alpha 1 \beta 3$ integrin.

CELL SIGNALLING PATHWAYS

Because of the complexity of intracellular signalling and, in particular communication between the various signalling pathways, it has been difficult to pinpoint the influences of cell adhesion molecule mediated from non-cell adhesion molecule mediated events. However, some have been analysed and revealed potentially interesting interactions between cell adhesion molecule mediated processes and others. For instance, the integrin signalling pathways also converge with those through which growth factor effects are signalled. When the $\alpha 5 \beta 1$ integrin binds to fibronectin, molecules of the mitogenic pathway, such as ras, associate with the integrin signalling complex.⁸¹ There is every chance that the interplay between different regulatory proteins and the integrins permit a coordinated response to multiple environmental factors.

A similar situation is found in the direct regulation of cell adhesion molecule expression. Adhesion molecules play an important part in the regulation of immune responses. Part of that regulatory mechanism involves the upregulation of expression of cell surface adhesion molecules. An example is the E-selectins (ELAMs), which are important mediators of inflammatory reactions and are upregulated within hours by inflammatory mediators such as tumour necrosis factor (TNF) and certain interleukins.

Cytokines released in other situations mediate similar effects. In normal epidermis $\alpha 2 \beta 1$, $\alpha 3 \beta 1$ and $\alpha 6 \beta 4$ are strictly confined to the basal layers of keratinocytes, being concentrated at their basal surfaces, abutting the basement membrane. In damaged skin, however, integrins are found around the periphery of basal and suprabasal cells. It is believed that in

this situation cytokines released from the damaged tissue lead to the altered distribution of integrins during re-epithelialisation.^{82–83} Similarly, ICAM-1 expression is inducible by lipopolysaccharide (endotoxin) as well as interleukin-1 and TNF α , which both synergise with interferon τ . It has been shown that a major difference between non-inflammatory osteoarthritis and inflammatory rheumatoid arthritis (RA) synovium is greater expression of ICAM-1 on RA macrophages. The significance in terms of disease processes, however, is unclear.⁸⁴

GENE CONTROL

Such is the key role of cell adhesion molecules in cell migration, proliferation and differentiation, it comes as no surprise to pathologists to find that these molecules are either encoded, or regulated, by genes disturbed in cancer. Many are proto-oncogenes. E-cadherin expression is regulated by the ErbB2 proto-oncogene⁴ and RHAMM is upregulated in H-ras transformed cells. In addition, various components of intercellular junctions have molecular structural similarities or close physical associations with products of tumour suppressor genes. For instance, there is evidence for a tumour suppressor function for E-cadherin (the tumour suppression gene product APC binds to β -catenin which is also cytoplasmically associated with E-cadherin⁶⁸) and the neurofibromatosis 2 tumour suppressor gene product merlin is a member of the moesin-ezrin-radixin family of junctional proteins.⁸⁵ Conversely, products of oncogenes such as src, ras, fos, and met have been demonstrated to destabilise intercellular junctions,⁸⁶ src (the gene encoding epidermal growth factor receptor) and met through phosphorylation of β -catenin at tyrosine residues.⁸⁷

Most exciting, at least to one interested in skeletal morphology, is to find that cell adhesion molecule expression is regulated by the morphoregulatory homeobox genes Hox and Pax (fig 6).⁸⁸ Now that the promoters for a number of cell adhesion molecules have been described, it is clear that certain sequences in these molecules are targets for homeobox gene products. It has been shown in mouse cells that the N-CAM promoter can be stimulated by the Hox-B9 product, a process that can be inhibited by the product of Hox-B8.⁸⁹ In the chicken, the L-CAM cadherin promoter has also been shown to be stimulated by a homeobox gene Hox-D9. Here too there is evidence of reciprocal feedback between genes that control cell adhesion and histogenesis, and genes that regulate spatial patterning in embryos.

Regulation at the genetic level can be controlled in other ways. For example, CD44 can display heterogeneity through alternative splicing of certain of its exons, resulting in different isoforms with differing binding capacities and hence physiological roles. These include lymphocyte homing, immune response regulation (through lymphocyte activation) and cell migration.

Summary

The cell adhesion molecules are ubiquitous recognition molecules that allow cells to communicate with one another and their environment. Through these molecules, complex alterations in the cytoplasmic messenger pathways and the microfilamentous cytoskeleton can lead to profound alterations in cell division, differentiation, behaviour, and function. It is difficult to conceive of a group of molecules that could be more important to pathologists and their understanding of disease processes.

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