REVIEW ARTICLE

Cellular Adhesion Molecules in Neurology

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ABSTRACT: The study of cellular adhesion molecules offers crucial understanding of cellular interactions. Their name implies an underestimation of their function, as intercellular glue. In fact, they play vital roles in tissue development and intra- and intercellular signaling. In neurology, cellular adhesion molecules are already providing welcome new insight into neurodevelopmental anomalies, autoimmune demyelination, and invasive tumours. Cellular adhesion molecule manipulation has led to several therapeutic options which are the subject of ongoing clinical investigation.

RÉSUMÉ: Les molécules d'adhésion cellulaire en neurologie. L'étude des molécules d'adhésion cellulaire (MACs) fournissent des connaissances cruciales sur les interactions cellulaires. Leur nom comporte une sous-estimation de leur fonction comme adhésif intercellulaire. En fait, elles jouent des rôles vitaux dans le développement des tissus et la signalisation intra et intercellulaire. En neurologie, les MACs fournissent déjà des éléments nouveaux dans la compréhension des anomalies du développement, de la démyélinisation auto-immune et des tumeurs invasives. La manipulation des MACs a ouvert la voie à plusieurs options thérapeutiques qui font l'objet présentement d'investigations cliniques.

Can. J. Neurol. Sci. 1997; 24: 200-209

The research on cellular adhesion molecules (CAMs) in neurodevelopment and neuropathology will have a major impact on theories of disease pathogenesis and perhaps treatment. Cell adhesion molecules interact with a ligand on another cell surface or within the extracellular matrix. They confer phenotype and behaviour on a cell. Behaviour results from signal transduction, allowing a neuron, for example, to respond with alteration of neurite outgrowth, cell migration, growth cone behaviour and synapse formation. In neurodevelopment, a vast neural network is generated and adhesion molecule-mediated interactions lead to distinct spatial differentiation. Unfortunately, the extensively differentiated elements of this complex network are restricted in their plasticity and are sensitive to insult. It follows that an understanding of cellular interactions will have important implications for the treatment of both developmental diseases and diseases that infiltrate the network.

CAM research is shedding light on mechanisms of abnormal neuronal migration and myelination and acquired CNS-specific disease. Some of the corresponding clinical expressions of deranged migration and myelination might include mental retardation, primary epilepsy, peripheral and possibly central dysmyelination. In MS, the pathological immune response might be targeted by CAM manipulation rather than exposing the patient to systemic immunosuppression. In neurooncology, specific CAMs are likely responsible for the behaviour of invasive infiltrating tumours and neurotropic metastases.

Based on structural homology, several families of adhesion molecules are recognized: the immunoglobulin (Ig) superfamily, cadherins, selectins, and integrins[1] (Table 1). After a brief introduction to these families, this paper will review the current data on the normal function of CAMs in the nervous system and their particular significance in multiple sclerosis and neurooncology.

The Immunoglobulin Superfamily of CAMs

Immunoglobulin (Ig) CAMs are characterized by at least one Ig fold of 60 to 100 amino acids providing the active site of adhesion[1,2] (Figure 1). The anchor consists of a transmembrane segment and a cytoplasmic tail. The founding member of this family is the 5-fold containing neural cell adhesion molecule (NCAM). Other nervous system specific Ig CAMs include L1 and the adhesion molecule on glia (AMOG) which function in neural migration, and PMP-22, P_0, and MAG which function in myelinization. The nervous system specific IgCAMs are distinguished from most other Ig CAMs by their homophilic interactions. Elsewhere, Ig CAMs abound on T-cell and endothelial cell surfaces and interact heterotypically. They include the cluster of differentiation antigens (CD), lymphocyte function antigens (LFA-2 and -3), and vascular and intercellular CAMs (VCAM and ICAM).

In the immune system then, the pairing for some of these heterotypic interactions is known. The expression of ICAM and VCAM on endothelium promotes the adhesion of integrin-expressing leukocytes giving the leukocytes an opportunity to detect a tropic stimulus.[3,4] Even the stimulation of T-cells by

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RECEIVED JULY 17, 1996. ACCEPTED IN FINAL FORM FEBRUARY 5, 1997.

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Table 1: Cell Adhesion Molecule Families.

Immunoglobulin supergene family
T-cell receptor (TCR)
Clusters of differentiation (numerous, e.g., T-cell: CD4, CD8. All cells: CD44)
Major histocompatibility complex (MHC) molecules
Lymphocyte function antigens (LFA-2 and its ligand, LFA-3)
Intercellular and vascular CAMs (ICAM, VCAM)
Neural CAMs (NCAM, L1, AMOG)
Myelin associated CAMs (P_0, PMP-22, MAG)
Cadherins
Types N, E, and P
Selectins
Lymphocyte homing receptor (L-selectin)
Endothelial leukocyte adhesion molecule (E-selectin)
Platelet-activation dependent granule-external membrane protein (P-selectin)
Integrins
Very late antigens expressed after T-cell activation (VLAs, and LFA-1)
Also referred to by alpha and beta subunit typing (e.g., $alpha_5$ $beta_1$)

way of the trimolecular complex is considered an adhesion molecule interaction. The interaction between antigen-presenting cell and T-helper cell is further stabilized by LFA-2 and LFA-3 binding, these CAMs being common to both cell types.

CD 44 was originally recognized as a lymphocyte homing molecule. Its expression on lymphocytes permits targetting to venular endothelium within lymph nodes. It is also found on epithelial cells and glial tumours where its expression is associated with more malignant behaviour.

Post-translational modification and altered gene expression provide means of changing the function of a given Ig CAM through the ontogeny of the organism. This will be reviewed in the specific case of NCAM.

Cadherins

These molecules jut out from a cell surface with five tandem repeats ready to bind a counter-poised cadherin on another cell surface.[1,6,7] Cadherins dimerize and are thereby capable of dual interactions: the binding proceeds in a zipper-like linear fashion. Binding is calcium-dependent, but also requires specific intracytoplasmic binding.

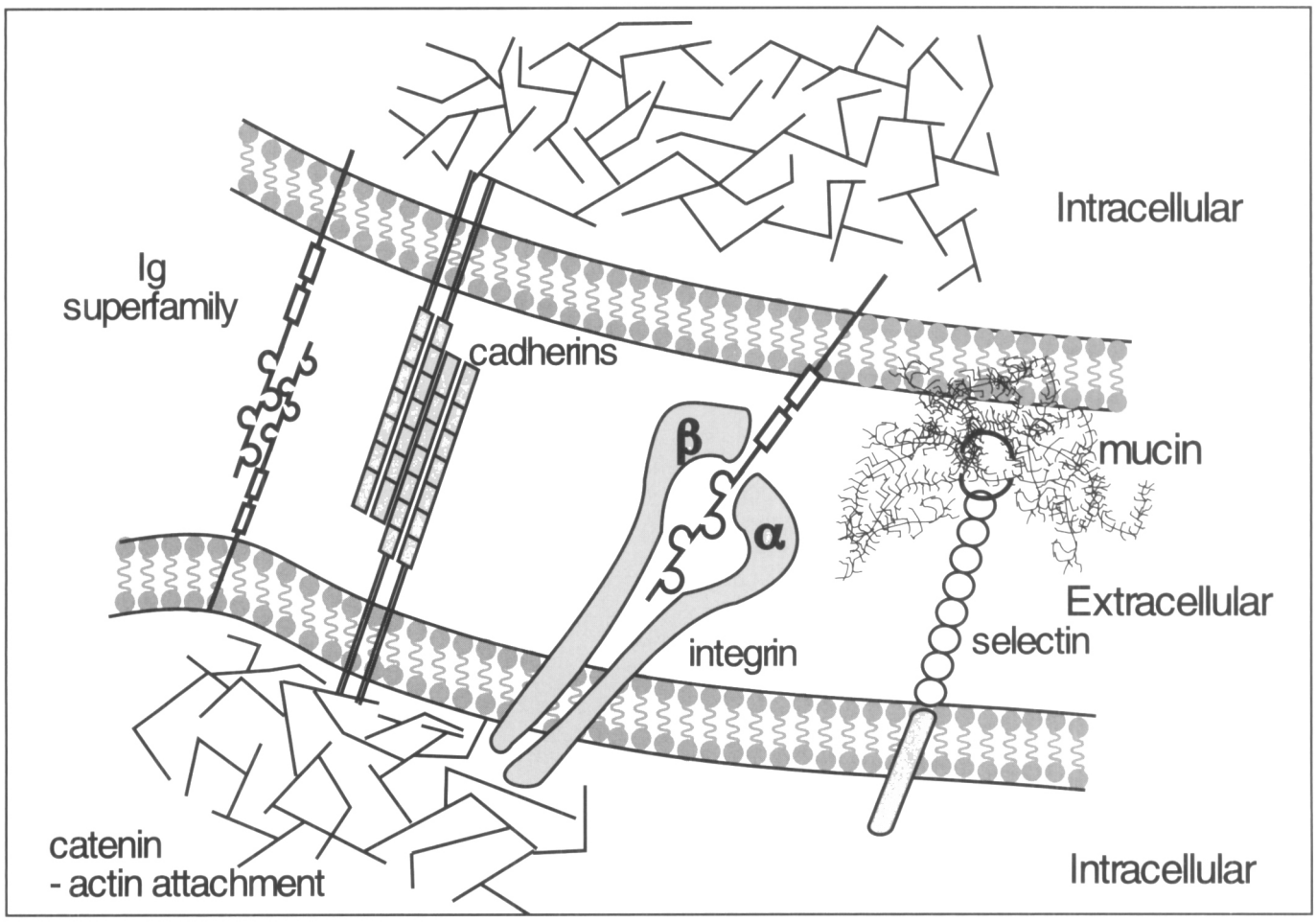

Figure 1: *Cellular Adhesion Molecules. Members of the Ig superfamily contain a variable number of Ig and fibronectin type III domains. Ig domains are depicted as loops joined by a disulfide bond. Variability within the Ig domains is generated by random alternative splicing and post-translational modification. Rectangles represent the fibronectin repeats. Cadherins are identified by ectodomain repeats and a cytoplasmic domain which binds to the cytoskeleton. The extracellular domains are homophilic. Selectins are mucin-like molecules which bind carbohydrate ligands and are responsible for leukocyte rolling and capture. Integrins are subclassified by their alpha and beta subunits. They bind to Ig CAMs and components of the extracellular matrix.*

https://doi.org/10.1017/S0317167100021806 Published online by Cambridge University Press

The cadherin is anchored to the cell membrane via a single transmembrane segment and a cytoplasmic carboxy-terminal domain. The intracytoplasmic domain binds catenins which further bind the cytoskeleton. Both calcium and catenins are requisites for cadherin-mediated adhesion. Tyrosine phosphorylation of beta-catenin diminishes adhesion as well and provides a site for phophatase-mediated cell regulation.[2]

Cadherins are subdivided into 1) N, P, R, B, and E cadherins which interact with actin-cytoskeleton, and 2) desmosome-associated cadherins which interact with intermediate filaments, contributing to the structure of tight junctions.[2]

Selectins

Selectins present an amino-terminal domain for a calcium-dependent interaction with sialylated glycans.[3,8] The extending arm includes a preterminal epidermal growth factor (EGF)-like domain and several peptide repeats. The selectins are abbreviated L-E-, and P-, for their respective localizations to the surfaces of lymphocytes, endothelial cells, and specific platelet granules. Their function in slowing the intravascular flow of leukocytes will be reviewed in the scheme of transendothelial migration.

Integrins

These glycoproteins are made up of two subunits, alpha and beta, with at least 15 different alpha and 8 different beta subunits to combine.[1,2] The active adhesion site lies within the beta subunit. Ligand adhesion requires an intact cytoplasmic tail for the beta subunit, and may be modified by metal ion binding to the alpha subunit. Integrin ligands are specific to a single Ig CAM, e.g., LFA-1/ICAM-1 or VLA-4/VCAM-1, or a subset of extracellular matrix (ECM) components.

Neuronal Cell Migration, Differentiation, and Plasticity

During normal development, neuroblasts migrate in an amoeboid fashion to and fro along radial glial processes, while continuing in the cell cycle. They divide only upon returning to the germinal matrix. Eventually, the cell stops dividing and then, partly differentiated, migrates a predetermined distance to aggregate with similar neurons. Its differentiation is subsequently influenced by axonal pathfinding, target recognition, and synaptogenesis.[9]

Axon-substrate interactions provide adhesion for the growth cone to proceed in response to a tropic stimulus. The adhesive interactions themselves also likely influence differentiation. Target associated signals will further specify neuronal phenotype even before a synapse is made. Final differentiation requires synapse formation. Even then, apoptosis may occur if the created circuitry is inactive or inappropriate to the cell type. There is some evidence that synapses are stabilized and transmission facilitated by polymerization of cellular adhesion molecules within the synapse.[10]

Intracellular Signaling

Cellular differentiation and expression of surface molecules result from the cell's perception of its environment. Its immediate physical environs is detected through CAMs. The repertoire of CAM expression is in turn restricted by the cell's differentiation. Several signaling pathways are known, but their complex interactions and intracellular compartmentalization account for the difficulty in manipulations at this level.

Integrin signaling partly occurs via a focal adhesion-associated kinase,[8,11] $pp125^{FAK}$. This should be akin to growth factor receptors which transduce signal by way of a tyrosine kinase. Ligand binding induces receptor clustering and autophosphorylation of tyrosine residues. The Ras-activator protein is brought to the receptor where Ras is activated through phosphorylation of GDP. Ras then stimulates several kinases. Some specificity may be afforded by intracellular organization.

The kinase, $pp125^{FAK}$, undergoes phosphorylation of tyrosine residues in response to integrin clustering. The extracellular domains of tyrosine kinases also share sequence homology with CAMs.[11] Notably, Src- and Fyn-deficient neurons have specific impairment of neurite outgrowth in response to L1 and NCAM-140 respectively. Fyn- deficient mice have impaired myelination which may be explained by a possible interaction with MAG.[11] Local cytoskeletal assembly is likely influenced and integrin binding in myoblasts is essential for myogenic differentiation.[8] The loss of integrin interaction in certain cell lines results in the form of programmed cell death known as apoptosis. Specific adhesion to fibronectin or anchored monoclonal antibodies to the beta-1 subunit is required to block the apoptosis of endothelial or epithelial cell lines.[8] The tumour necrosis factor (TNF) receptor activates a sphingomyelinase, releasing ceramide from sphingomyelin. The ceramide activates a signaling pathway which has also been implicated in apoptosis.

G-proteins are associated with peptide hormone signal transduction. After ligand binding, they induce physically associated enzymes to generate second messengers such as cyclic-AMP, diacyl glycerol (DAG), or inositol triphosphate (IP_3). The activation results from the binding of GTP to the altered alpha subunit of the G-protein. Both NCAM and N-cadherin induced neurite outgrowth are dependent on G-proteins.[8,12] The subsequent activation of N- and L-type calcium channels reflects the intracellular localization of the G-protein activated cascade.

The cytoskeleton itself may influence intracellular signaling pathways.[13] Changing the tension on fibroblasts alters cAMP concentrations. Conversely, the structure and dynamics of the cytoskeleton can be altered by adjacent cell membrane interactions.

The Growth Cone

At the terminus of the growth cone, filopodia are protruded and are believed to function as an autonomous sensory unit to direct growth orientation.[14] Filopodial movements include protrusion, lateral sweeping and retraction. The mechanism of these movements is calcium-dependent and NCAM, N-cadherin, and L1 mediate fluxes of calcium in the filopodia. The associated cell contact-dependent neurite outgrowth can be completely inhibited by N- and L-type calcium channel antagonists.[15,16] Arachidonic acid (AA) will mimic the neurite outgrowth response stimulated by CAMs[16]. Doherty and Walsh proposed a model for signal transduction following the homophilic interaction of CAMs wherein the interaction of adhesion molecules would result in the synthesis of AA. Neurite outgrowth induced by nerve growth factor (NGF), protein kinases A or C occurs by a different mechanism. It is unaffected by modulation of the voltage dependent calcium channels, L and N, or extracellular calcium concentration.

homophilic binding. The mechanism of intercellular adhesion is calcium independent and is inhibited by sialylation, a means of post translational modification. The extent of sialylation of NCAM is threefold greater in embryos than in adults, contributing 30% of the molecule's mass.

Sialylation not only reduces the binding activity of NCAMs, but also enhances the neurite outgrowth response. The removal or blocking of polysialic acid residues by enzymes or antibodies respectively results in disrupted neural tube formation,[17] defects in neural crest cell migration and inhibition of motor axon outgrowth in chick embryos.[18,19] The sialylation of NCAM has been measured in certain primary CNS tumours, but has not correlated consistently with tumour behaviour.[20]

NCAM-dependent neurite outgrowth is also impaired by increased use of the so called Variable Alternatively Spliced Exon (VASE) in NCAM synthesis.[21] Again, this loss of neurite outgrowth induction is associated with increased NCAM binding activity in vitro. Diminished post-translational sialylation and increased VASE incorporation are therefore implicated in the changing role of CAMs to promote neurite outgrowth in early development and thereafter to stabilize synaptic connections and inhibit plasticity.

Mutations of Ig Superfamily CAMs

The importance of Ig superfamily CAMs in nervous system development has been explored using null mutations. In mice, NCAM knockout produces only a mild phenotype with underdevelopment of the olfactory bulb and a lack of rostral migration of granule cells into the bulb.[22] This was surprising given the presumed significance of NCAM in early neurodevelopment, and it suggests that compensatory molecules exist.

L1 is a member of the Ig superfamily that affects neurite outgrowth, fasciculation, migration, and synapse formation. Three mutations in the human L1 gene have been identified. The corresponding diseases are X-linked hydrocephalus due to stenosis of the Sylvian aqueduct (HSAS),[23,24] a form of hereditary spastic paraplegia (SPG1),[24] and congenital mental retardation with aphasia, shuffling gait, and adducted thumbs (MASA).[24,25] What remains unknown is the specific pathogenesis of each syndrome as it relates to the respective mutated domains.

The adhesion molecule on glia (AMOG) exhibits cell type-specific binding to neurons and increases neurite outgrowth. It is in fact the beta2 subunit of the enzyme Na/K-ATPase and, like its homologue the beta1 subunit, tightly associates with the alpha subunit. Expression begins in late embryonic stages, and is maximal in the mature adult brain. Knockout mutant mice behave normally until two weeks of age when they experience a rapidly progressive neurodegenerative disorder. The bulk of disease is in the diencephalon and brainstem with the spongiform appearance of swollen axons and glia and prominent vacuoles. It is unknown whether the pathology represents failure of the molecule's function in cell-cell interaction or as part of an ion pump. One would expect that AMOG functions in a recognition-mediated triggering of ion pump activity in the maturing CNS.[26]

Homophilic interactions of the myelin protein P zero (P_0) and peripheral myelin protein-22 (PMP-22) function in myelin compaction in the PNS.[26-28] A P_0 null mutant mouse has hypomyelinated nerves with a phenotype known as the *shiverer* mouse. In humans, point mutations of the P_0 gene on chromosome 1q are responsible for the distal neuropathy of the CMT-1/HSMN-I and more severe congenital Dejerine-Sottas/HSMN-III phenotypes. A duplication mutation of the PMP-22 gene on chromosome 17q similarly causes a CMT-I phenotype and a point mutation has been responsible for a Dejerine-Sottas phenotype. Each of these diseases is characterized by onion bulb pathology with multiple rings of myelin revealing a history of repeated demyelination and remyelination. A deletion mutation of PMP-22 causes a hereditary hypertrophic neuropathy with susceptibility to pressure palsies, also known as tomaculous neuropathy. *Tomacula* refer to focal sausage-like enlargements of myelinated axons which consist of redundant loops of myelin. That a mutation of an inter-Schwaan cell gap junction also causes a CMT-1 phenotype suggests a relationship between P_0 or PMP-22 and gap junction formation in the formation and maintenance of myelin.[29]

Myelin-associated glycoprotein (MAG) similarly belongs to the Ig superfamily and affects neurite outgrowth and adhesion of oligodendrocytes and Schwann cells to neurons. In MAG-deficient mice, the mutant phenotype may be subtle.[30] In the CNS, there is disorganization of the periaxonal cytoplasmic collar and hypermyelination. In the PNS, structure and function are unchanged perhaps because NCAM functions as a surrogate adhesion molecule. There is some speculation that MAG confers additional stability to the maintenance of myelin, but remains to be established in aging knockout mutants.[27]

Alternative mRNA splicing introduces a specific limitation to the utility of null mutation experiments for the Ig superfamily. Different forms of CAMs from the same gene are expressed at different times and sites. Neuroglian, for example, has cytoplasmic domains of different lengths, depending on its expression in the central or peripheral nervous system.[31]

Cadherins

Disaggregation of neural tissue may also be induced by the administration of antibodies to N-cadherin. The cadherins derive from multiple distinct genes. Again, the mechanism of intercellular adhesion is homophilic, but calcium-dependent. It is also dependent on the binding of the cytoplasmic domain to a family of proteins called catenins which interact with the cytoskeleton.[6,7] Expression of N-cadherin begins at the time of neural induction and is maintained after neural differentiation.[6]

Integrins

The integrins are glycoproteins comprised of two noncovalently associated subunits, alpha and beta, and were initially characterized by their expression on T-cells. They bind either to ECM or to members of the Ig superfamily.[22] In the extracellular matrix, fibronectin is secreted by mesenchymal cells and provides an adhesive surface for migrating neural crest cells and regenerating axons.[32]

Targeted gene inactivation of the integrins has emphasized the important role they play in developmental processes.[22] Beta-1, alpha-4 or alpha-5 result in early embryonic death. At this very early stage, CNS development was undisturbed. Efforts are being made to restrict these experiments topographically at various ontogenic stages to explore specific effects on nervous system development. Beta-1 antisense retrovirus has been constructed for local injection. Antibodies and antisense oligonucleotides might be similarly used to interfere with ECM ligands.[22]

Extracellular matrix (ECM)

Altered expression of ECM components will affect the function of their integrin ligand. They are not CAMs per se since they are not anchored to cell membranes. Congenital hypogonadism from deficiency of gonadotrophin releasing hormone and anosmia associated with the absence of olfactory bulbs occur together in an X-linked disorder known as Kallman Syndrome. The presumed migrational defect relates to neuroblasts within the olfactory placode. The responsible gene produces a protein known as KAL which shares properties with other ECM glycoproteins.[33]

Merosin is an isoform of the glycoprotein, laminin. It belongs to a group of dystrophin-associated glycoproteins which establish a structural link between sarcolemma and the cytoskeleton. As exemplified in Duchennne's and Becker's muscular dystrophies, the complex has proven itself essential to the myocyte's structural integrity, and merosin, specifically, has been implicated in Fukuyama congenital muscular dystrophy.[36]

S-laminin, another laminin isoform, has been inactivated by gene targeting to produce a perinatally lethal phenotype. The presynaptic membrane of the neuromuscular junction has a markedly diminished number of active zones.[22] The concurrent finding of poorly developed post-synaptic junctional folds is likely secondary. A more subtle mutation of this gene may be implicated in various forms of congenital myasthenia.

There is reason to believe that ECM molecule accessibility as a CAM ligand is modified by its association with other ECM molecules, although the interplay among CAM systems remains largely unexplored.

Multiple Sclerosis (MS)

MS is believed to be an autoimmune disease. Demyelination in multiple sclerosis occurs in conjunction with perivascular lymphocytic infiltrates. The diagnosis requires clinical or paraclinical evidence of plaques of inflammatory demyelination disseminated in space and time. Axonal loss and incomplete remyelination are processes which limit the extent of recovery. The perivenular inflammation is characterized by an enlarging ring of CD4+ cells and myelinophagic macrophages/microglia with death of oligodendrocytes.

One of the most important early events in the pathogenesis of autoimmune demyelination is migration of the activated TH-cell across the blood-brain barrier. Its migration may be divided into three stages, known as the area code model[3,4] (Figure 2). The first stage is a weak attachment of the T-cell to the endothelium referred to as rolling and capture. The second is the upregulation and production of additional adhesion molecules, induced by cytokine release in the immediate vicinity. The third is diapedesis across the endothelium, as the T-cell follows chemotactic gradients. Treatment of MS with available immunosuppressive drugs has been generally disappointing and limited by systemic toxicity. Recent partial success with IFN-beta brings the promise of more refined immunomodulatory therapy.[35] Among its several immunemodulating effects, IFN-beta reduces the expression of proinflammatory cytokines such as IFN-gamma and IL-1. The latter are known to increase cell surface concentrations of adhesion molecules. CAMs play a crucial role in initiation and activation of the immune response. Furthermore, serum levels of soluble CAMs (sCAMs) may provide an alternate means of quantifying disease activity in the assessment of investigational therapies.

Initiation of the Immune Response

Immunosurveillance of the CNS by migrating T cells is a normal occurrence. Memory T-cells would be particularly adept at crossing the BBB because of their increased expression of L-selectin.[36] E-selectin is found exclusively on vascular endothelium,[3] and its interaction with sialylated glycans on leukocytes is the likely basis for T-cell rolling and capture on the endothelial surface. This initial interaction induces the expression of other adhesion molecules, particularly integrins.

The adhesion molecule systems described generally include an Ig superfamily adhesion molecule expressed by endothelium coupled with an integrin expressed by leukocytes. Of the integrins, VLA-4 has shown increased affinity to the endothelially expressed adhesion molecules VCAM-1 and ICAM.[37] Myelin basic protein-sensitized T-cells increase their expression of VLA-4, and antibodies to this integrin block the development of EAE.[38]

The mechanisms of normal immunosurveillance yield a pathological response when antigen-specific clones are recruited. TH-cell activation is mediated in part through the formation of the trimolecular complex of processed antigen in conjunction with an MHC molecules on the antigen-presenting cell and the TCR. In the CNS, astrocytes, microglia and perivascular cells are capable of antigen presentation. This interaction is specific to T-cells bearing the CD4 adhesion molecule, and antibodies to MHC class II will inhibit the development of EAE.[39,40]

Class II MHC is inducible in a population of macrophages and reactive microglia. However, even in the absence of T-cell infiltration, as occurs in chronic silent MS plaques, macrophages remain intensely immunoreactive for MHC class II. It is speculated that the prolonged expression of class II MHC makes these areas susceptible to recurrent inflammation.[40]

Apart from their function in cellular migration across the blood-brain barrier, adhesion molecules are also crucial in lymphocyte proliferation.[41] Lymphocytes taken from rats pretreated with anti-ICAM-1 monoclonal antibodies failed to proliferate in response to MBP. Furthermore, LFA-2 and LFA-3 interactions promote T-cell proliferation independent of TCR stimulation. So, binding not only facilitates contact between T-cells and antigen-presenting cells, but provides costimulatory signals for T-cell activation.[42-44]

The adhesion molecule profile of the MS plaque

The initial impetus for MS research into adhesion molecules was to identify CNS-specific molecules crucial to the pathogenesis of MS. Broznan et al. suggest that molecules expressed in inflammation in the CNS are no different from those in peripheral inflammation. In their neuropathology series, TNF-alpha was centrally focussed in the perivascular MS lesion, localizing primarily to macrophages.[45] IFN-gamma expression on perivascular inflammatory cells was prominent, but did not correlate with lesion age. IL-1 was expressed by macrophages both perivascularly and at the expanding margin of the lesion, suggesting a role for it as the forerunner of cytokine expression in immune activation.

In all inflammatory CNS lesions, high expression of adhesion molecules was present, but a specific pattern in MS could not be identified. The ICAM-1/LFA-1 system was ubiquitous in plaques of all ages. VCAM-1/VLA-4 was expressed in established active and chronic silent plaques. These post mortem

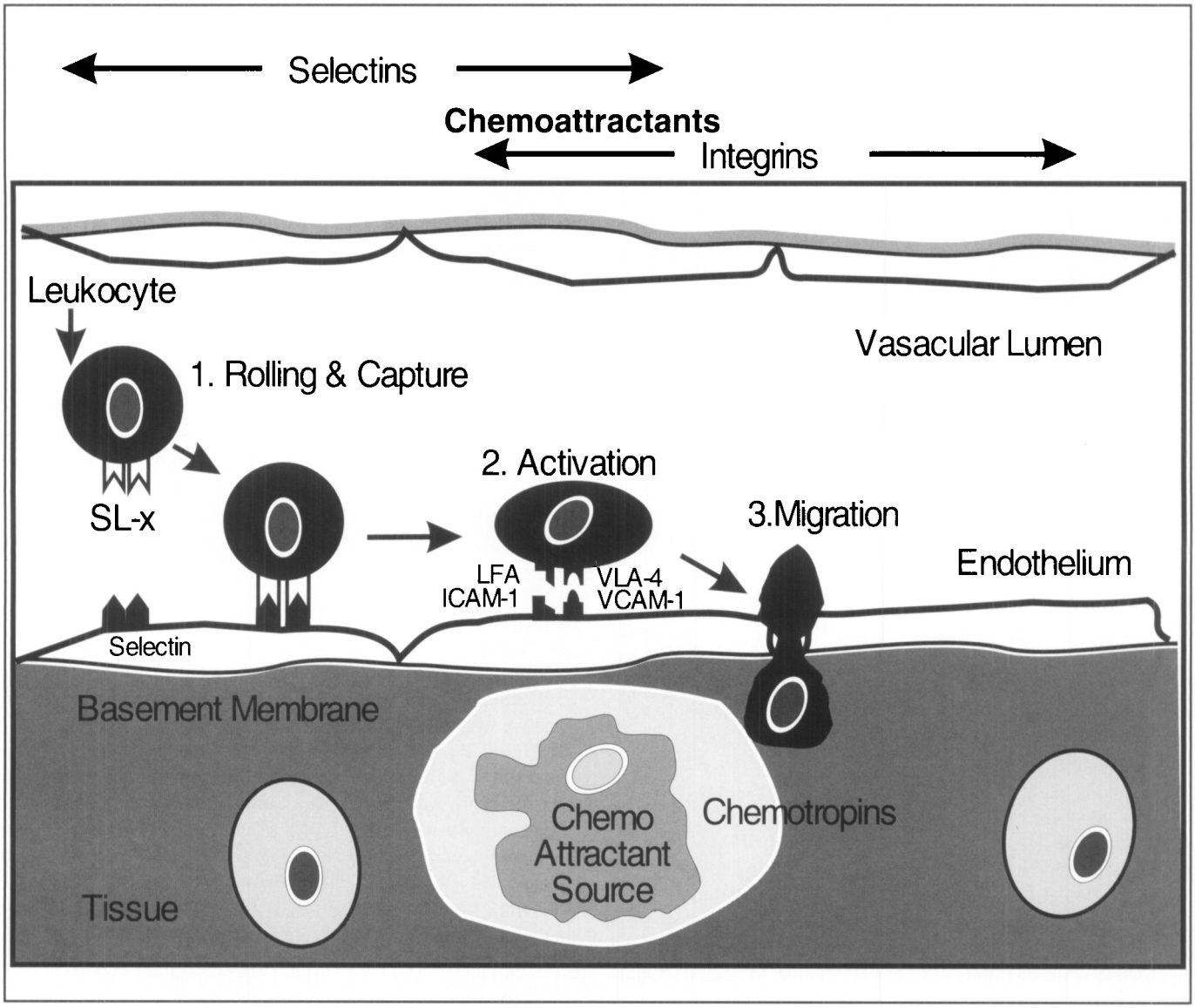

Figure 2: *Transendothelial leukocyte migration occurs in three steps. 1. Initial rolling and capture is mediated by selectin binding to carbohydrate, typically sialylated Lewis-x-like carbohydrates. 2. Capture results in activation by cytokine release and increased expression of the ICAM-1/LFA-1 and VCAM-1/VLA-4 systems promotes adhesion. 3. Migration occurs in the direction of highest chemoattractant concentrations.*

findings are consistent with in vitro experiments demonstrating induction of adhesion molecule expression in response to pro-inflammatory cytokines (IL-1, TNF-alpha, and IFN-gamma).

Experimental allergic/autoimmune encephalitis is an animal model for MS wherein the animal is sensitized to myelin basic protein. Administration of monoclonal antibodies against adhesion molecules has effected stabilization of active disease with anti-ICAM-1,[41,46] prevention of disease with anti-VLA-4 antibodies,[38] but a more severe course of disease with anti-LFA-1.[46,47] As suggested by the temporal sequence of adhesion molecule expression, the response to the injection of monoclonal antibodies in EAE may be highly dependent on the timing of their administration.[48]

Increased leukocyte adhesion to endothelial cells with upregulation of ICAM-1 demonstrated with IFN-gamma, TNF-alpha, and IL-1 in vitro.[4] TNF also induces expression of its receptor (TNFR). Shedding from cell surfaces produces a peak in plasma levels of the soluble receptor (sTNFR) by four weeks after a relapse.[49] sTNFR may be capable of antagonizing TNF action in vivo. In the chronic relapsing model of EAE, disease was improved by administration of sTNFR.

The targeted depletion of lymphocytes can be accomplished by use of monoclonals against specific CAMs. Some early clinical trials have already yielded promising results using anti-CD4[50] and anti-CDw52[51] to deplete lymphocyte populations. The number of active lesions on gadolinium-enhanced magnetic resonance images decreased in both study populations. More aggressive pan-lymphocyte depletion with OKT3 is limited by systemic toxicity and opportunistic infections.[52] Plans are underway for clinical trials using monoclonals against VLA and TCR.[53]

In the search for more specific immune modulation, transforming growth factor-beta has some possibilities.[54] The effects of this

https://doi.org/10.1017/S0317167100021806 Published online by Cambridge University Press

cytokine differ depending on the cell type, its degree of differentiation and its location. It is a chemotactic factor for T-cells, but inhibits their proliferation in short-term cultures. The primary immune response is also be abated by the suppression of E-selectin seen with TGF-beta. TGF-beta may suppress a primary immune response, but may then induce a secondary antigen-specific one. Before CD4 cells differentiate into Th1 or Th2, TGF-beta can induce them to become memory cells. It also enhances MHC II expression on B-cells, promoting their function in antigen presentation. However, the multiple effects of TGF-beta must be further clarified: it suppresses MHC II expression on melanoma cells, suggesting a means for tumours to evade immune surveillance.

The inhibition of transendothelial leukocyte migration by interfering with CAM function is also showing promise in stroke models. Monoclonal antibodies to CD11b/CD18, expressed by monocytes/macrophages reduce infarct volume in rat stroke, liver ischemia, and canine myocardial ischemia models.[55,56] The adhesion of *Bordatella pertussis* to monocytes is mediated through the interaction of CD11b/CD18 and the bacterial filamentous hemaglutinin (FHA). Oligopeptides of key FHA fragments have been used with similar efficacy as monoclonals to inhibit transendothelial migration in stroke models.

Soluble adhesion molecules in MS

CAMs are ultimately shed from the cell surface. The detection of soluble adhesion molecules may be valuable in quantifying the activity of inflammatory disease and marking its temporal progression. This has important ramifications in therapeutic trials where the clinical estimate of disease activity can be difficult. In this regard, MRI of the brain has proven helpful but remains time consuming and costly and correlates weakly with clinical scales of disability.

Preliminary data of CSF levels of soluble ICAM-1 has shown correlation with disease activity in multiple sclerosis (MS).[36] Although high levels of ICAM and VCAM have been observed in other inflammatory and degenerative neurologic diseases,[45] the value of their measurement would be in quantifying disease activity rather than in establishing diagnosis. The finding that CSF T-cell expression of VLA-4 in MS is less than in normal controls was unexpected, since its ligand is VCAM-1 and monoclonal antibodies to VLA-4 abrogate EAE.

In a study involving 147 patients with MS,[48] soluble CAM levels correlated modestly with MRI assessment of active disease: 0.47 for sL-selectin, 0.36 for sVCAM-1 and 0.28 for sICAM-1. The lack of better correlation may reflect the temporal sequence of adhesion molecule expression and the uncertain kinetics of their shedding and clearance.

Soluble adhesion molecules may retain functional activity. Physiological levels of L-selectin have shown ability to impair lymphocyte adhesion to endothlial cells in vitro.[57]

CAMs in Neurooncology

Equally tantalizing is the research of cell surface molecules in CNS neoplasms. Of the gliomas, astrocytomas in particular extensively infiltrate the surrounding brain, making them refractory to surgical management. The purpose of adhesion molecule research in this field lies in clarifying the mechanisms of tumour infiltration, neurotropic metastasis, and the immune response incited against neoplasms.

Tumour invasion and metastases in the CNS

Invasion may be mediated by adhesion molecules in cell-cell or cell-ECM interactions. In comparison with other tissues, brain ECM is a relatively amorphous matrix of glycosaminoglycans. In the process of invasion by tumour, the ECM is digested by secreted hydrolytic enzymes and tumour cells migrate onto a proteolytically modified matrix. The rarity of extracranial metastases of the primary CNS tumours argues for their dependence on a specific migrational substrate.

In part, this may be attributable to an over expression of the cell's basic phenotype. For example, CD44 mediates adhesion to the ECM through hyaluronate binding and is expressed on several cell surfaces. In normal brain, glial expression of CD44 occurs especially on perivascular astrocytes within the white matter. In the cortex, glia do not express CD44,[58] while in high grade astrocytomas and glioblastoma multiforme, it is strongly expressed. However, the level of CD44 expression fails to correlate with tumour grade.[32,59] More recently, splicing variants of CD44 (CD44v) have been recognized as metastasis-promoting factors. All of 56 primary brain tumours examined by Li et al. expressed standard CD44, while 22 of 26 metastases expressed CD44v.[60]

Ariza et al. have demonstrated CD44 in all of 10 specimens of glioblastoma and only weak positivity in one of 10 meningioma specimens.[5] CD44v expression was absent in these non-metastasizing primary CNS tumours. The use of anti-CD44 monoclonals has resulted in decreased invasiveness of CD44-positive tumour cells in vitro.

The differential expression of CAMs can modify tumour behaviour and versatility. A relative reduction in cadherin/catenin interactions in epithelial tumours is associated with tumour invasiveness.[8] Of the integrins, lower expression of $alpha_5beta_1$ will decrease aggregability of transformed hamster ovary cells and enhance their migration.[61] Increased expression of $alpha_vbeta_3$ will also result in increased migration of melanoma cells,[62] and the expression of a VCAM-1 epitope, E-selectin, ICAM-1, and LFA-1 have also been correlated with the metastatic potential of non primary CNS malignancies.[63-65]

Integrin expression by primary CNS tumour cells has been correlated with tumour type and grade.[66] The consequences of integrin expression relate to the interaction between cell surface and the ECM. Tumour cell migration can be characterized by their preferred ECM substrates. Astrocytoma cells migrate on a variety of ECM subtrates while primitive neuroectodermal tumour (PNET) cells are restricted to laminin, fibronectin, or type IV collagen. Cell lines from high grade tumours are able to migrate on collagens more extensively than those from low grade tumours. The ECM subset characterizing PNET cell migration is richly expressed in the leptomeninges and suggests an explanation for the propensity of PNETs to seed the pia-arachnoid. The migration assay is nevertheless limited by the observation that cell phenotype gradually changes in vitro: while glioblastoma cell lines migrate well on collagens, cells from glioblastomas often perform poorly.

Astrocytomas richly express LFA-3, also known as $alpha_vbeta_1$,[65] and their in vitro migration is possible on a wider range of ECM media than PNETs.[66] In vivo, the preferred migrational substrate may be provided in part by reactive glia depositing fibronectin. Nevertheless, the integrin components chiefly

https://doi.org/10.1017/S0317167100021806 Published online by Cambridge University Press

responsible for the success of astrocytoma cell migration are $alpha_v$ and $beta_1$. Monoclonals raised against them will completely inhibit migration on most ECM substrates. Apoptosis was not observed in these experiments.

Melanoma cells lacking expression of $alpha_v beta_3$ integrin undergo spontaneous apoptosis when isolated from other tissue.[66] They are otherwise sustained by ectopic expression of the integrin. On the other hand, the loss of other integrins has been associated with the blocking of programmed cell death. In that situation, the donation of integrin by adjacent normal cells may induce appropriate deletion of tumour cells.

Tenascin expression shows a moderate correlation with astrocytoma grade and vascular hyperplasia in some studies,[67,68] but Friedlander et al. have had contrary observations.[66] Tenascin-c has also failed to show an essential function in animal studies with null mutations.[22]

NCAM expression may be associated with reduced tumour infiltration.[69] Transfection of a rat glioma cell line with human NCAM isoform resulted in the down regulation of proteases capable of digesting ECM. This suggests that increased NCAM expression results in decreased tumour invasiveness.

Immune response

The aim of this area of research is to enhance immune surveillance and response against tumour cells. Tumour infiltrating lymphocytes (TILs) are described in most solid tumours,[70,71] presumably responding to novel antigens. In the case of the CNS, the blood-brain barrier (BBB) establishes an immune privileged environment and, other than microglia which derive from monocyte lineage, leukocytes are generally excluded.

With tumour-associated angiogenesis there is rarely a complete BBB, the endothelial tight junctions being induced only by mature glia. This provides greater opportunity for lymphocyte infiltration. The mechanisms of rolling and capture must be involved and in fact, increased expression of E-selectin on intratumoural endothelial cells has been demonstrated.[32]

The reason for subsequent leukocyte activation remains unclear. Human glioblastoma cells may retain their function as antigen-presenting cells, and 40% of malignant gliomas express MHC class II molecules.[72] ICAM-1 expression may be an adequate surrogate for immune activation in the absence of MHC class II.[73] ICAM-1 and LFA-1 monoclonal antibodies inhibited TIL and LAK cell binding to human glioblastoma cells in vitro.[70] In a study of 12 glioma specimens, the lymphocyte infiltrate was more prominent in the seven which expressed ICAM-1.[74] There was no relationship between the extent of lymphocyte infiltration and clinical course of the gliomas in this small cohort.

Since VCAM-1/VLA-4 interactions have been implicated in lymphocyte migration, modulating VCAM-1 expression might have more immediate clinical implications. The upregulation of VCAM-1 expression on astrocytoma cells has been observed in response to combinations of IFN-gamma, TNF-alpha, and IL-2.[75] In vitro, LAK cells will increase ICAM-1 expression on vascular endothelium and tumour cells.[76] Additional stimulus may be offered by microglia, which being of monocyte derivation, are induced to secrete TNF-alpha and IL-1b upon engagement of LFA-3 and CD-44. TNF-alpha in concert with IFN-gamma provided the greatest stimulus for upregulation of VCAM-1, thereby to enhance the VCAM-1/VLA-4 interaction implicated in lymphocyte migration.

Unfortunately, even after administration of LAK cells and IL-2 into gliomas, the subsequent migration of effector immune cells was negligible. TILs are suppressed in their cytotoxic and proliferative capacities by antiinflammatory cytokines associated with tumours such as TGF-beta2 and PGE 2.[77,78] Furthermore, the direct effects of a given cytokine on astrocytoma cells can be either inhibitory or stimulatory depending on the specific array of cell surface antigens in the cell line.[79] Attempts at enhancing tumour infiltration by T cells and subsequent activation are still in their infancy.

Conclusions

CAMs offer insight into abnormal neural development. CAM interactions govern growth cone behaviour which exemplifies their dual roles of cell surface adhesion and signal transduction. The function of a given CAM will change based on the timing and localization of its expression. More ubiquitous adhesion molecules have proven essential in the development of multiple systems. In strong contrast, altered expression of nervous system-specific CAMs may produce only subtle neurodevelopmental abnormalities and varying degrees of dysmyelination from the myelin associated CAMs; therefore, the presence of surrogate CAM systems has been speculated.

In MS, memory T cells are implicated in the pathogenesis by virtue of high L-selectin expression. After capture on the endothelium, VLA-4 expression is increased and interacts with VCAM-1 and ICAM. Following migration into the CNS, antigen presenting cells interact with the lymphocytes and induce cytokine release. Cytokines in turn, induce antigen presentation and increase the expression of adhesion molecules. Lymphocytes proliferate and additional lymphocytes are recruited. As LFA expression increases, proliferation may be antigen independent. Microglia become myelinophagic and maintain a high expression of MHC class II, suggesting a perivascular region primed for recurrent or chronic inflammation. Successful immune modulation has been demonstrated with various monoclonal antibodies against CAMs, and assaying soluble CAMs may be useful in monitoring disease activity.

In the realm of neurooncology, NCAM may reduce the infiltrative behaviour of glial tumours; though alternatively, it may only reflect a greater degree of glial differentiation. CD44 expression may promote infiltration into the brain parenchyma, while its splicing variants are associated with the metastatic potential of non-primary CNS tumours. E-selectin, ICAM-1, and LFA-1 have been similarly implicated in determining the metastatic potential of certain malignancies. Tumour growth is also dependent on its vascular supply, and to that end, the role of CAMs in tumour associated angiogenesis is being pursued. Integrin expression also contributes to tumour behaviour, and blocking $alpha_v$ and $beta_1$ subunits may already suggest a therapeutic option. Finally immunosurveillance of tumour cells can be exploited. In spite of a population of TILs, the cells remain largely inactive. Their function in the tumour is unknown, but attempts are being made to enhance recruitment of peripheral immune cells and to activate existing TILs.

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