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Animal Lectins as Cell Adhesion Molecules

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Abstract
Protein-carbohydrate interaction is exploited in cell adhesion mechanisms besides the recognition of peptide motifs. The sugar code thus significantly contributes to the intriguing specificity of cellular selection of binding partners. Focusing on two classes of lectins (selectins and galectins), it is evident that their functionality for mediation of adhesive contacts is becoming increasingly appreciated, as is the integration of this type of interaction with other recognition modes to yield the noted specificity. The initial contact formation between leukocytes and activated endothelium makes use of selectins to guide lymphocyte trafficking. In addition to the three selectins which bind a distinct array of ligands, galectin-1 and galectin-3 and possibly other members of this family are involved in cell-cell or cell-matrix interactions. This review summarizes structural and functional aspects of these two classes of endogenous lectins relevant for cell adhesion.

Introduction

The ability of cells to adhere to each other or to constituents of the extracellular matrix (ECM) is fundamental for metazoan organisms. Adhesive phenomena are intimately involved in normal complex multicellular processes such as embryonic development, organogenesis, angiogenesis, and inflammation, and in aberrant processes leading to tumor invasion and metastasis. Actually, these reaction cascades are the object of intense research efforts aiming at the development of new antiadhesive therapeutic interventions. To be able to target distinct molecules, the panel of participating molecules and their structural details must be known. At first sight, the multitude of the individual compounds involved in cell adhesion mechanisms appears to be confusing [Joseph-Silverstein and Silverstein, 1998]. However, common themes enable to form categories which can then be subdivided. Focusing on the type of molecular interaction, not only protein-protein recognition is operative. As similarly emphasized by Sinowatz et al. [1998] and Mann and Waterman [1998] in this issue, another fundamental molecular interaction makes a pivotal contribution to the accuracy and versatility of cell interactions. Based on the enormous potential of sugar structures to code biologi-

Abbreviations used in this paper:
CIDNP = Chemically induced dynamic nuclear polarization; CRD = carbohydrate recognition domain; ECM = extracellular matrix; FAK = focal adhesion kinase; GlyCAM-1 = glycosylation-dependent cell adhesion molecule-1; ICAM = intercellular adhesion molecule; Ig = immunoglobulin; LAD I/II = leukocyte adhesion deficiency I/II; LFA-1 = lymphocyte function-associated antigen-1; LPAM-1 = lymphocyte Peyer's patch adhesion molecule-1; MAdCAM-1 = mucosal addressin cell adhesion molecule-1; NMR = nuclear magnetic resonance; SCR = short consensus repeat; TNFα = tumor necrosis factor-α; VCAM-1 = vascular cell adhesion molecule-1; VLA-4 = very-late-antigen-4.

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cal information [Laine, 1997] and on the presence of lectins as receptors for such structures in animal tissues [Gabius, 1991, 1997a; Zanetta, 1997, 1998; for a collection of recent reviews, see Gabius and Gabius, 1997], it has been evident that protein-carbohydrate interactions play a crucial role in recognitive events. Notably, the chemical details of this recognition are already being unraveled [Gabius, 1998; Lis and Sharon, 1998; von der Lieth et al., 1998]. Concerning the mode of binding between lectins and their appropriate carbohydrate ligands general principles can be discerned, as illustrated in figure 1. It is pertinent to note that this scheme is also relevant for lectins from other organisms [Jacobson and Doyle, 1996; Gilboa-Garber et al., 1997; Ward, 1997; Lis and Sharon, 1998; Rüdiger, 1998]. Carbohydrate epitopes for specific binding can be provided both from surface-associated glycoproteins or glycolipids on adjacent cells as well as from carbohydrate chains of adhesive proteins of the basal lamina and the ECM, respectively. Common structural parts of N-glycans of proteins are shown in the chapter of Geyer and Geyer [1998] in this issue. Even the sugar part of neutral glycolipids or gangliosides, structural details being presented by Hakomori [1998] in this issue, can serve as ligand, as convincingly proved for ganglioside GM_1 recently [Kopitz et al., 1998]. In addition to direct binding of complementary carbohydrate motifs, the 'bridging mode' to cross-link either carbohydrate structures or lectins is a common binding type. The formation of complexes with distinct stoichiometry is considered to be prerequisite for the initiation of ensuing signaling events which can trigger a variety of postbinding events [Gupta et al., 1996; Villalobo and Gabius, 1998]. When complementary surfaces of carbohydrates are placed in juxtaposition, carbohydrate-carbohydrate interaction can also occur [Bovin, 1997].

This review will introduce the reader to two main families of animal lectins and their activities in this respect illustrated by graphic examples. Based on the established category formation of animal lectins by the molecular architecture of the carbohydrate recognition domain (CRD) five families have been defined, C-type lectins, galectins and I-type lectins being primarily relevant in this context [Powell and Varki, 1995; Gabius, 1997a]. C-type lectins are characterized by their dependence on the presence of Ca^{2+} ions for sugar binding and the preservation of a common sequence motif of 14 invariable and 18 highly conserved amino acid residues [Drickamer, 1993; Sharon, 1993; Weis and Drickamer, 1996; Gabius, 1997a]. In this context, the members of the selectin subgroup of C-type lectins deserve special attention, as they participate in the early events leading to leukocyte extravasation.

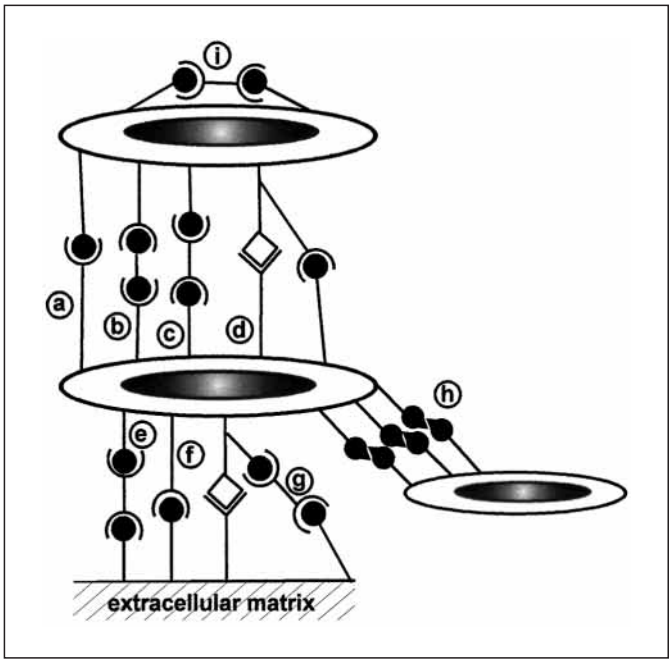


Fig. 1. Schematic drawing of theoretical possibilities for cell surface interactions involving oligosaccharides and lectins. Two cells can associate by presentation of lectin-glycoligand pairs on apposing surfaces such as direct binding between a lectin and its appropriate carbohydrate ligand (a). A further binding mode either engages a bi- or multivalent lectin which is capable to bridge carbohydrate ligands on the cell surface of neighboring cells (b) or soluble bi- or multivalent carbohydrate ligands (c) which can cross-link lectins which are noncovalently associated with the cell surface or anchored with a transmembrane domain in the cell membrane. A peculiar mode of action is given in (d). Besides being engaged in protein-carbohydrate interaction the lectin can also bind to a protein ligand on the apposing cell membrane, establishing a two-point association favored by different modules in the lectin sequence. Glycobiological interactions with molecules of the basal lamina and the extracellular matrix are represented by types (e–g). Either the bridging mode (e) or the direct binding mode (f) result in the adhesion of cells to constituents of the basal lamina (e.g. laminin) and/or the ECM (e.g. fibronectin). The situation in (g) reflects the possibility for independent protein-protein and protein-carbohydrate interactions involving the principal binding mode (e), i.e. the binding of a dimeric lectin cross-linking the carbohydrate chains of cell surface proteins, which are also involved in protein-protein interactions with oligosaccharide chains of matrix proteins. Thus, the strength of the interaction can readily be enhanced to effect tight binding. If clusters of complementary carbohydrate surfaces are suitably located, adhesion can also be mediated by carbohydrate-carbohydrate interactions (h). Bridging is not restricted to different cell surfaces, but can also occur between ligands on the cell surface of the same cell (i). As currently known, the binding type in situation (a) is preferred e.g. by C-type lectins such as selectins, several galectins and I-type lectins. Galectins of the proto- and tandem-repeat type such as galectin-1 or galectin-8 appear capable of mediating their effects via the modes illustrated in (b, e–g, i).

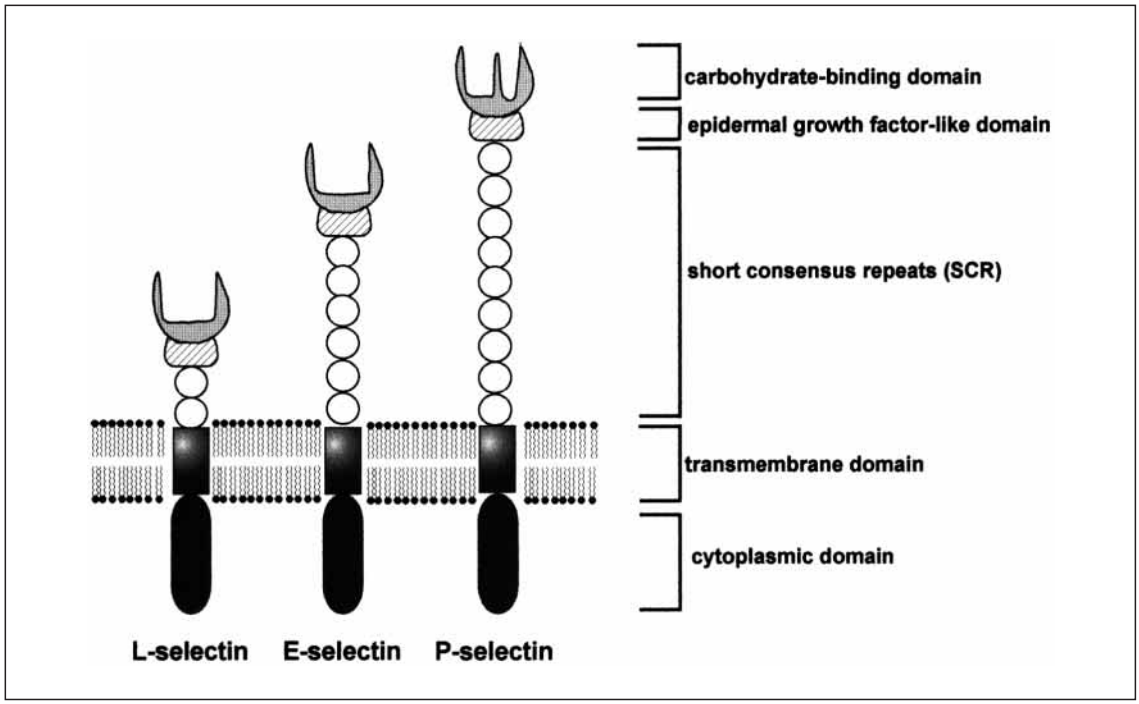


Fig. 2. Structural characteristics of selectins. The modular arrangement of the three family members starts with an extracellular N-terminal lectin domain and an epidermal growth factor-like region. Two (L-selectin), six (E-selectin), and nine (P-selectin) consensus repeats homologous to a complement-binding domain, a transmembrane region and a cytoplasmic C-terminal domain complete the modular arrangement of these type I transmembrane proteins.

Selectins

Initially, the first selectin had been detected as antigen of a monoclonal antibody inhibiting lymphocyte homing [Gallatin et al., 1983]. The members of this subgroup of adhesion receptors were then identified on activated endothelial cells (E-selectin), leukocytes (L-selectin) and activated platelets (P-selectin) [Bevilacqua and Nelson, 1993]. These receptors exert their function by forming an initially loose contact between leukocytes and endothelial cells during the early phase of inflammation. Endpoint of the ensuing process cascade is the recruitment of immune cells to extravascular tissue to destroy pathogens, pathogen-infected cells, and cell debris. The selectins structurally share a common modular arrangement of domains which is illustrated in figure 2. The three members of this family are type 1 transmembrane proteins and their extracellular region is composed of an amino-terminal calcium-dependent lectin domain (CRD), an epidermal growth factor-like motif and a varying number (2–9) of short consensus repeat (SCR) units, which are homologous to domains found in complement-binding proteins [Lasky, 1995; Nelson et al., 1995; Tedder et al., 1995]. This peculiar arrangement of the three modules only occurs in selectins, although each of these motifs can be found in other proteins such as proteoglycan core proteins [Iozzo and Murdoch, 1996; Gabius, 1997a]. It guarantees that the terminal CRD is ideally positioned to recognize appropriate glycoligands presented on an apposing cell surface. To be able to bind to a determinant on another cell surface, the binding site of the lectin must extend beyond other molecules. Especially, the peculiar molecular arrangement with six SCR segments in the case of E-selectin and nine in the case of P-selectin leads to the projection of the CRD into the bloodstream allowing optimal accessibility for ligands on adjacent cells. Concerning carbohydrate ligands sialylated and fucosylated lactosamines had been found to be critical for selectin binding. Such features are assembled for example in the tetrasaccharide sialyl Lewis^x and its structural variants (fig. 3). The way how the tetrasaccharide motif 'decorates' the protein or lipid scaffolds determines the actual ligand potency of the glycoconjugate [Varki, 1994]. Notably, the presently known ligands for L- and P-selectin belong to the mucin family (fig. 4A; structural depictions in this figure and

forthcoming illustrations are explained in detail in fig. 4B). Members of this family are characterized as long rod-like glycoproteins with multiple serine and threonine residues resulting in heavily O-glycosylated domains. The interaction between appropriate O-glycans and L-selectin is a factor in the adhesive function of L-selectin, involving the cytoplasmic tail's capacity to associate with calmodulin [Kahn et al., 1998]. Similar to the analogous section of for example the epidermal growth factor receptor [Martin-Nieto and Villalobo, 1998], this part is also a salient interaction partner for calmodulin. However, recent findings suggest that apart from the mucin-associated moieties sialyl Lewis^x-like L-selectin ligands exist which are resistant to O-sialoglycoprotease treatment [Clark et al., 1998]. Besides their presence an additional factor is suggested to determine their suitability to serve as ligands. The formation of patches of selectin-reactive oligosaccharides may well be of relevance to create high-affinity in vivo ligands, as discussed in detail by Varki [1997]. Low affinity of binding and a very fast dissociation constant for monomeric L-selectin/GlyCAM-1 interaction argue in favor of the importance of clustering on cell surfaces [Nicholson et al., 1998]. In this environment, the location and unique characteristic structural features renders selectins and their ligands suitable to establish contacts necessary for cells to roll along the luminal site of the vessel wall and to tolerate shear forces. The elongated molecular structures of selectins, especially in the case of E-selectin (six SCR modules) and P-selectin (nine SCR modules) and mucins together with their segmental flexibility are predicted to enhance their accessibility for binding to counterstructures on closely opposed cells.

Following this brief survey, further details on each of the three selectins will be presented. L-Selectin is constitutively expressed on the surface of lymphocytes preferentially at the tips of the microvilli. This distinct positioning facilitates that L-selectin can optimally interact with its ligands on adjacent cells despite the short stretch of SCR segments (only two). Besides its occurrence on the surface of neutrophils contributing to the process of extravasation, L-selectin is involved in the homing of T cells [Rossiter et al., 1997]. The homing process is defined as tissue-selective trafficking guided by organ-specific adhesion molecules on specialized endothelial cells. Whereas in peripheral lymph nodes soluble glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1) [Lasky et al., 1992] and transmembrane CD34 [Baumhueter et al., 1993] were identified as possible L-selectin ligands [Tedder et al., 1995; Crottet et al., 1996], binding to mucosal addressin cell adhesion molecule 1 (MAdCAM-1; see fig. 6) was reported in (mesenteric) mucosal lymph nodes and gut Peyer's patches [Berg et al., 1993; Briskin et al., 1993]. The mucin-like protein GlyCAM-1 is synthesized by specialized endothelial cells of high endothelial venules, devoid of a membrane spanning domain and secreted into the blood [Brustein et al., 1992; Dowbenko et al., 1993]. Whether GlyCAM-1 promotes or inhibits L-selectin-mediated adhesion depends on the cell type. For example, ligation of L-selectin via GlyCAM-1 enhances β_2 integrin function in naive, but not in memory T cells [Hwang et al., 1996]. The mucin-like CD34 transmembrane molecule is concentrated in filopodia of nonspecialized endothelial cells found in the microvasculature of most tissues [Springer, 1995]. Regarding Peyer's patches MAdCAM-1, a dual functional role is reported. Initially, lymphocytes are attached to the mucosal addressin via L-selectin and tetrasaccharide epitopes on the O-linked oligosaccharide chains of the mucosal addressin. Actual rolling additionally involves the binding to $\alpha_4\beta_7$ integrins via peptide motifs of the immunoglobulin-like domains [Bargatze et al., 1995]. Compared to L-selectin the other two members of this lectin group are not only expressed by blood cells.

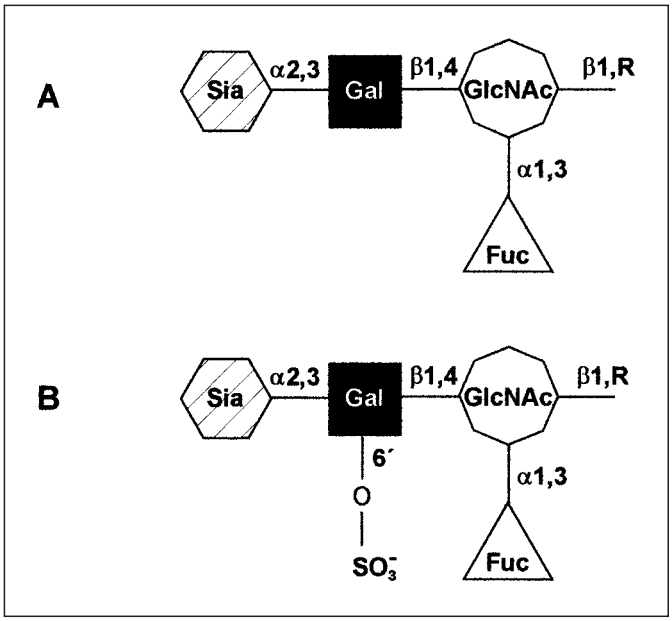


Fig. 3. Structure of the sialyl Lewis^x (**A**) epitope and the 6′-sulfated derivative of sialyl Lewis^x (**B**). Sialyl-Lewis^x is a tetrasaccharide motif shared by selectin ligands and can be recognized by the CRD of all selectins. The sulfated form is a determinant displayed on carbohydrate chains of GlyCAM-1. Fuc = *L*-Fucose; Gal = *D*-galactose; GlcNAc = N-acetyl-*D*-glucosamine; Sia = N-acetyl-*D*-neuraminic acid.

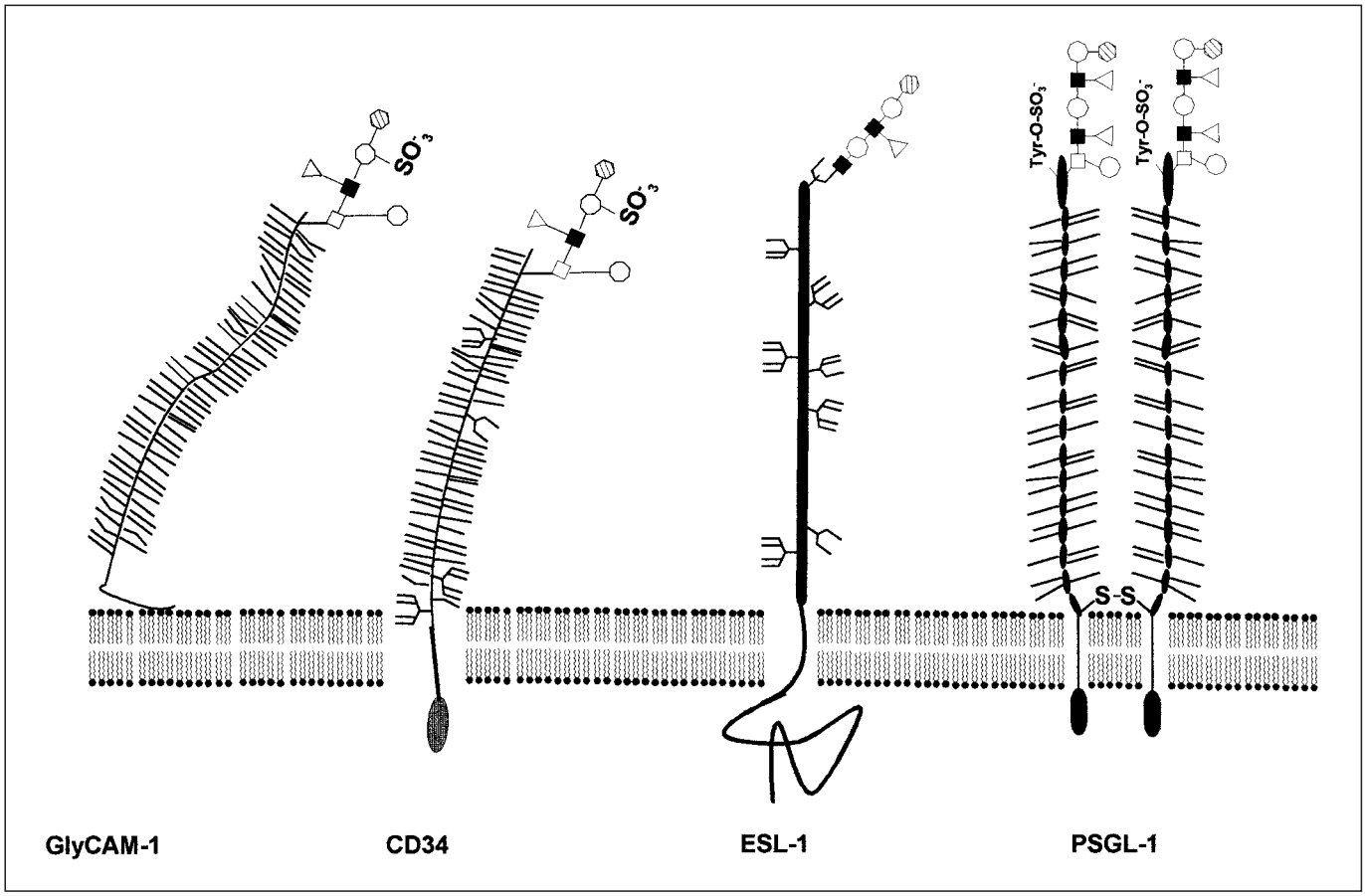
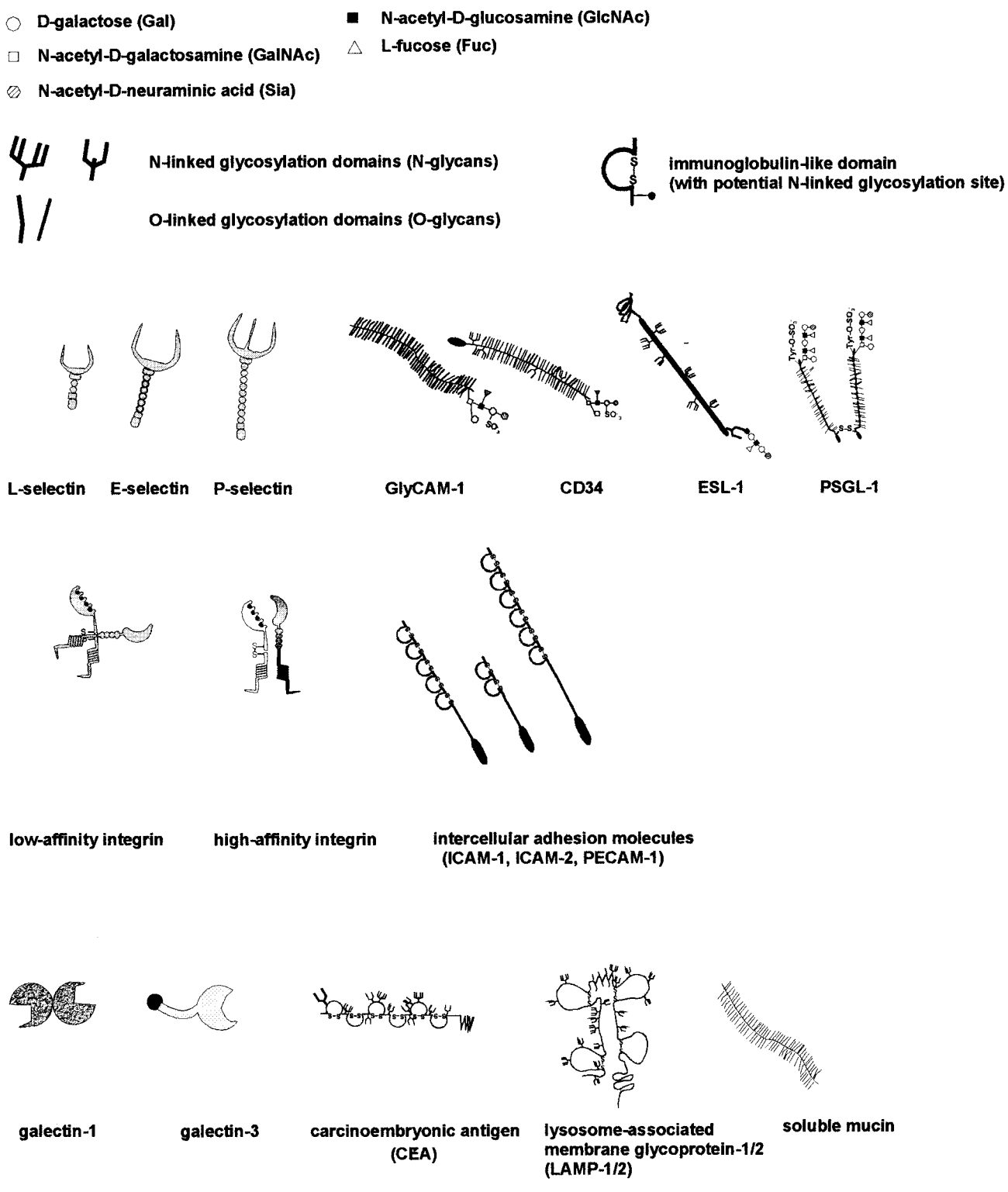


Fig. 4. **A** Schematic illustration of natural selectin ligands, carrying carbohydrate motifs outlined in figure 3. GlyCAM-1 or CD34, two heavily O-glycosylated mucin-like proteins, are in vitro ligands of L-selectin. Both are constitutively expressed on high endothelial venules of peripheral lymph nodes. GlyCAM-1 lacks a transmembrane domain and is most probably a secretory protein, whereas CD34 is a type 1 transmembrane glycoprotein of 140 amino acids. The protein is widely distributed in different tissues. However, presentation of the glycosylation suitable for selectin binding is restricted to special anatomic sites. ESL-1 is a proposed ligand for E-selectin discovered by direct affinity isolation [Levinovitz et al., 1993] and expression cloning [Steegmaier et al., 1995]. In contrast to the mucin-type ligands this glycoprotein is only scarcely decorated by N-glycans. P-Selectin glycoprotein ligand-1 (PSGL-1) is a disulfide bridge-linked dimeric type 1 transmembrane protein of 402 amino acids isolated from human leukocytes. The extracellular part is composed of 16 decameric repeats. Three NH_2-terminal tyrosines at positions 46, 48 and 51 are potential sulfation sites. PSGL-1 is a mucin-like protein rich in serines and threonines as potential O-glycosylation sites bearing O-glycans with sialylated and fucosylated poly-N-acetyllactosamine sequences $[\text{-3Gal}\beta\text{1-4GlcNAc}\beta\text{1-}]_n$ [reviewed by McEver and Cummings, 1997]. To deliberately illustrate structural details of crucial sites on the glycan part, their density and arrangement on the carrier and the backbone itself in one figure, the sizes of the individual parts of the scheme cannot be true to scale. **B** Key to structural depictions in **A**, figures 6, 7 and 10.

The expression of E-selectin is restricted to activated endothelial cells [Bevilacqua et al., 1987]. Various classes of triggering molecules like interleukin-1, tumor necrosis factor-α (TNFα) or lipopolysaccharide released at inflammatory sites lead to a transient de novo expression of E-selectin mRNA. The peak of this transcriptional activation is reached about 4–6 h after stimulation and declines within 24–48 h to basal levels. Compared to L-selectin and P-selectin ligands, E-selectin ligand-1 identified by direct affinity isolation [Levinovitz et al., 1993] and cloned from mouse myeloid cells [Steegmaier et al., 1995] is not a sialomucin (fig. 4). Instead, it employs one or more of its five potential N-linked carbohydrate side chains for ligand activity. Furthermore, skin-homing T cells expressing cuta-



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neous lymphocyte antigen bind to E-selectin expressed on venule endothelial cells in skin [Berg et al., 1991]. The third member of this C-type subgroup, namely P-selectin, found on the surface of activated platelets and of endothelial cells, is capable of mediating platelet-leukocyte and also endothelial cell-leukocyte interactions. P-Selectin is stored in α-granules of platelets and in the Weibel-Palade bodies of endothelial cells and is released from these intracellular compartments upon activation. As the cytoplasmic region of P-selectin harbors phosphorylation sites, rapid phosphorylation and dephosphorylation occur upon cellular activation supporting the view of a function of P-selectin in cellular signaling, as further discussed by Crockett-Torabi [1998] and Villalobo and Gabius [1998]. With respect to ligand recognition, P-selectin interacts with recognition points on the tetrasaccharide motif, but its natural mucin-like ligand P-selectin glycoprotein ligand-1 contains also an N-terminal sequence stretch with sulfated tyrosine residues which is involved in the interaction with the P-selectin's CRD (fig. 4A). Coexpression of both the oligosaccharide and the tyrosine sulfates improves the association rate resulting in high on-rates [McEver et al., 1995; Pouyani and Seed, 1995; Sako et al., 1995; Wilkins et al., 1995; McEver and Cummings, 1997].

As already mentioned, *L*-fucose is an indispensable part of the carbohydrate motifs sialyl-Lewis^x and its derivatives, which possess ligand-binding properties for the members of the selectin subgroup. This reasoning would receive compelling support, if defects of accurate fucosylation within glycan assembly [for comprehensive review on enzymology of glycosylation, see Brockhausen and Schachter, 1997] can be referred to. Indeed, when carbohydrate structures in patients are devoid of *L*-fucose as a consequence of a congenital disorder of endogenous fucose metabolism, symptoms of leukocyte adhesion deficiency II (LAD II) syndrome occur [Etzioni et al., 1992; Frydman et al., 1992]. In addition to the expected strong immunological effects such as recurrent infections, poor wound healing or persistent leukocytosis patients have also neurological defects, craniofacial dysmorphism, and exhibit the rare Bombay blood group phenotype [Paulson, 1996]. The correlation of the immunological manifestations to this natural defect is corroborated by the targeted disruption of the genes for P- and E-selectin, as outlined in Frenette and Wagner [1997] and Gabius [1997a]. Further examples of how aberrations in glycan chain synthesis are linked to human disease are expertly compiled and discussed by Brockhausen et al. [1998] in this issue.

Having up to this point illustrated structural aspects of selectins and their ligands and discussed the primary event of contact formation between leukocytes and endothelial cells, it is instructive to describe how this step is integrated into the complete cascade of processes to yield cell adhesion and extravasation.

Selectins and Integrins: Orchestrated Interplay in Leukocyte Adhesion

Integrins are a family of heterodimeric glycoproteins composed of two non-covalently associated subunits which mediate contacts to matrix proteins. As shown in figure 5, the two types of subunit can combine from a panel of α- or β-chains, yielding a variety of different α,β-combinations. To date, 22 heterodimers composed of 16 different α subunits and 8 different β subunits can be distinguished. Although the ligand-binding site is found on the β-subunit, both subunits are essential to recognize the adhesive ligand with their extracellular parts. The complex is also required to establish the interaction of the cytoplasmic domain of the β subunit with the cytoskeleton, as illustrated in figure 5. Aggregation or occupancy of integrin molecules upon activation organizes intracellular proteins into adhesion (supramolecular) complexes and induce the activation of signaling molecules which regulate cell morphology, migration, gene expression, growth and differentiation. These hierarchical and synergistic molecular interactions are mediated through the subunits' cytoplasmic domains, for example direct binding interactions with the β_1-subunit have been reported for talin, α actinin and focal adhesion kinase, as shown in figure 5. Especially the multifunctional tyrosine kinase FAK mediates the cross-talk between cytoskeletal and signaling molecules providing binding sites for a remarkable number of these molecules [Yamada and Geiger, 1997]. Moreover, calreticulin seems to be a modulator of cellular adhesiveness through direct interaction with the cytoplasmic tail of the α subunit of integrin molecules [Dedhar, 1994; Krause and Michalak, 1997].

Integrins are widely distributed and occur on the surface of different cell types such as leukocytes, epithelial and endothelial cells, myocytes, fibroblasts and osteoblasts, implying diverse roles in various physiological adhesion processes. The capability of integrins to contact their ligands is cation-dependent and based on their inherent binding capacity to specific sequence stretches of the ligand protein such as the RGD motif (arginine-glycine-aspartate). Since a detailed discussion of integrins and their ligands is outside the scope of this review, the reader is referred to recent reviews on this topic [e.g. Ruoslahti, 1996; Brown, 1997; Gahmberg et al., 1997; Bazzoni and Hemler, 1998; Schlaepfer and Hunter, 1998]. In this context it is sufficient

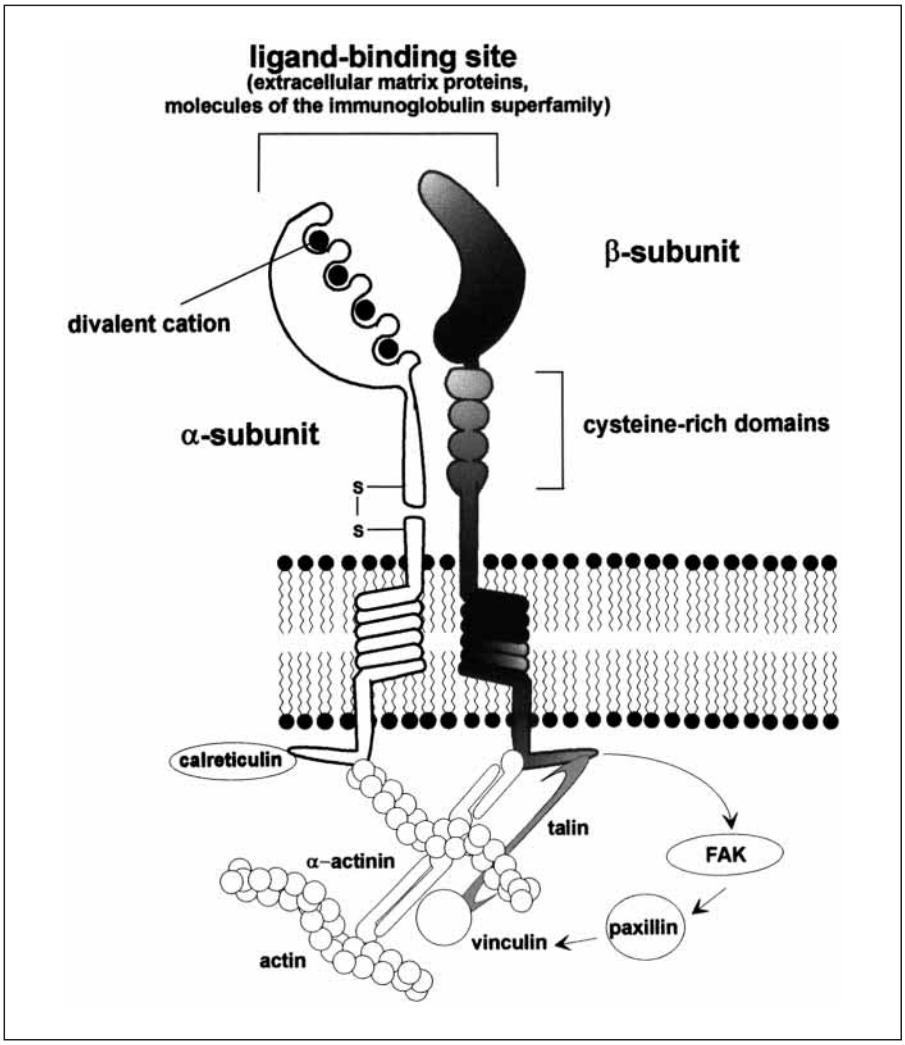


Fig. 5. Schematic drawing of characteristic structural details of an integrin. Integrins are heterodimers constituted by an α- and a β-subunit. The extracellular part of the α-subunit is folded into four divalent-cation-binding domains, the extracellular part of the β-subunit consists of cysteine-rich domains. Integrins are transmembrane proteins capable of recognizing with their extracellular parts short peptide sequences either of matrix proteins (e.g. laminin, fibronectin) or of immunoglobulin-like adhesion molecules (see fig. 6). Binding processes can be relayed to the cell's cytoskeletal and signaling molecules via the cytoplasmic tails which can directly contact e.g. in the case of the β_1-subunit to talin, α-actinin or focal adhesion kinase (FAK) or in the case of the α-subunit to calreticulin.

to point out that at least five of these integrins are involved in the interaction of leukocytes with the vascular endothelium. The β_2 integrin $\alpha_L\beta_2$ (CD11a/CD18), also known as lymphocyte function-associated antigen-1 (LFA-1), is expressed on all leukocytes. The integrins $\alpha_M\beta_2$ (CD11b/CD18) or Mac-1, and $\alpha_X\beta_2$ (CD11c/CD18) or p150/95 are found on blood neutrophils and monocytes. In addition, the two α_4 integrins, namely $\alpha_4\beta_1$ (CD49d/CD29) or very-late-antigen-4 (VLA-4) and $\alpha_4\beta_7$ or lymphocyte Peyer's patch adhesion molecule-1 (LPAM-1), are found on lymphocytes and some other leukocytes. The main ligands for leukocyte integrins belong to the immunoglobulin superfamily (intercellular adhesion molecule, ICAM). This family consists of more than 70 members and is the largest group of surface receptors representing a significant portion of the adhesion molecules on leukocytes, thrombocytes, endothelial cells and fibroblasts. Their extracellular part is composed of one or more repeats of immunoglobulin (Ig) folds, each 60–100 amino acids long with two β-sheets covalently linked by disulfide bridges, as shown by a selection of Ig fold-containing family members (fig. 6). LFA-1 binds to the ICAMs, ICAM-1 and ICAM-2, expressed on endothelial cells [Gahmberg et al., 1997]. Mac-1 and p150/95, which have overlapping specificities, can bind numerous ligands including ICAM-1 on endothelial cells as well as C3bi-coated particles, fibrinogen, and factor X. VLA-4 binds to the ICAM vascular cell adhesion molecule-1 (VCAM-1), and the CS-1 fragment of fibronectin, while $\alpha_4\beta_7$ has been shown to bind to VCAM-1 and to MAdCAM-1.

As already indicated, selectins are involved in the primary recognition. Since leukocyte extravasation occurs after a series of steps, further molecules are required. Only

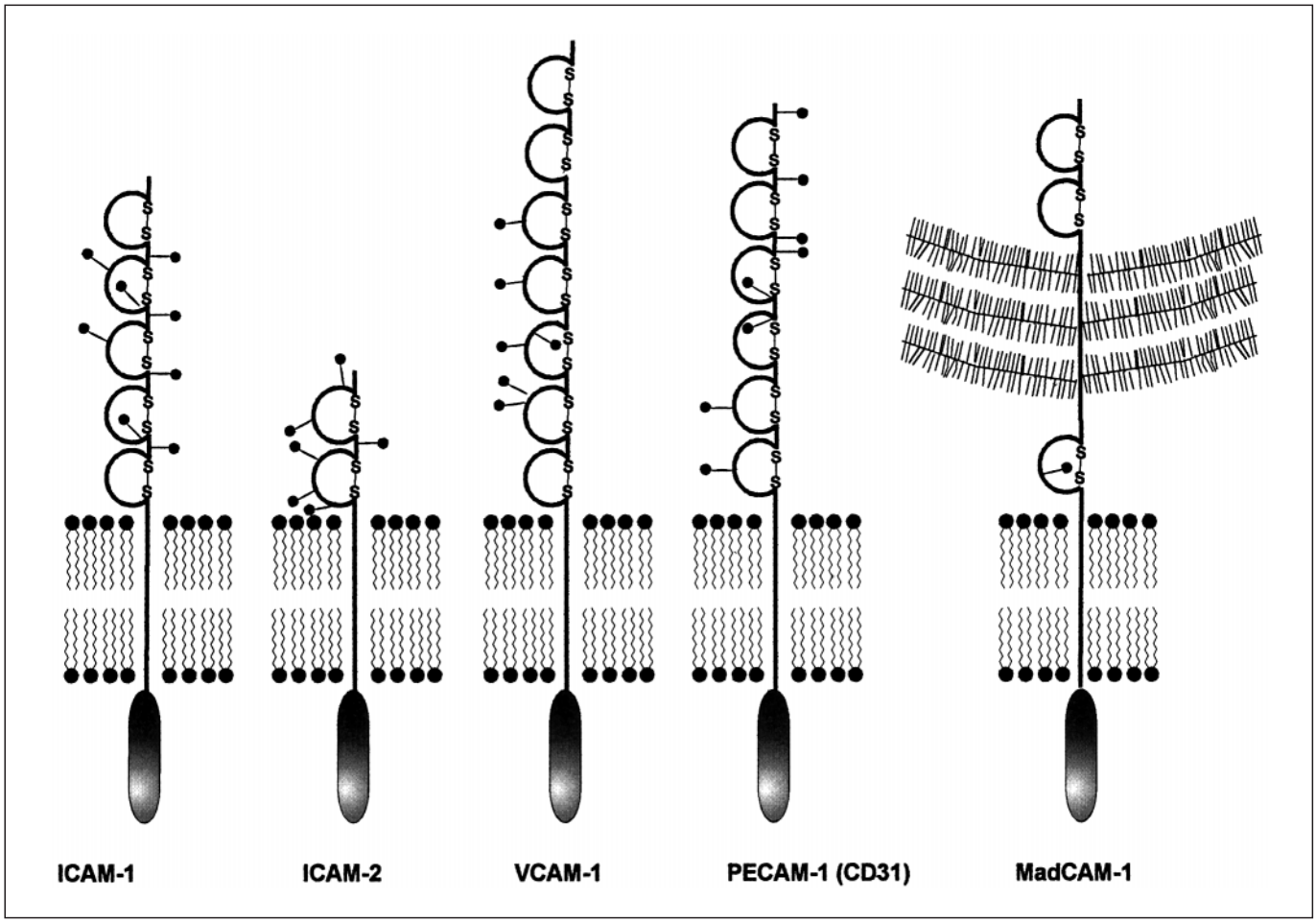
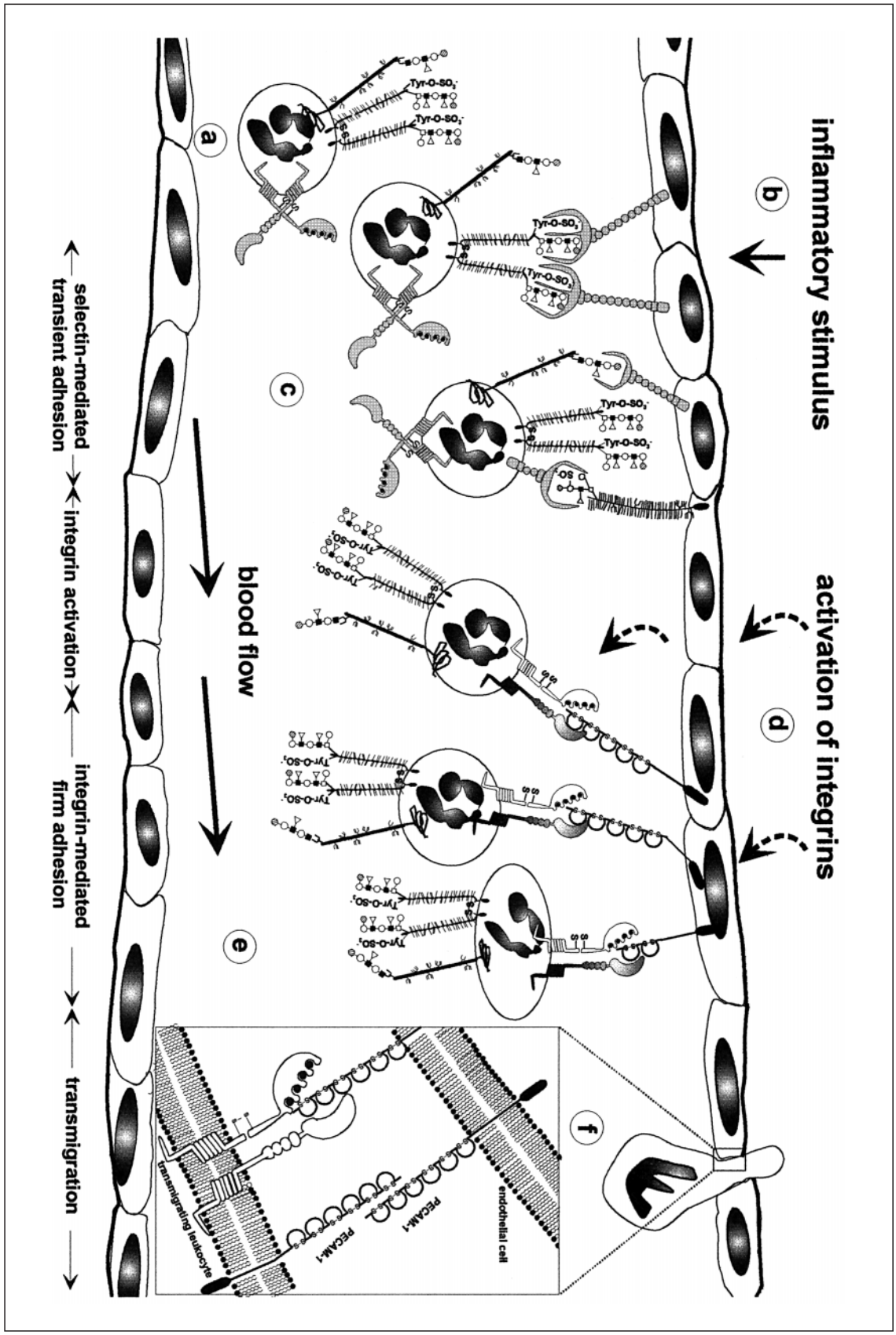


Fig. 6. Selected members of the immunoglobulin superfamily relevant for cell adhesion. Leukocyte-endothelial interactions can involve ICAM-1 and ICAM-2, VCAM-1, MadCAM-1, and platelet and endothelial cell adhesion molecule 1 (PECAM-1; CD31). The basic motif, the external immunoglobulin fold, is composed of between 70 and 110 amino acid residues containing two β-sheets. The β-strands are held together by a highly conserved disulfide bridge [Gahmberg et al., 1997]. MAdCAM-1, originally purified from mesenteric lymph nodes, contains immunoglobulin-like domains, providing peptide motifs for the $\alpha_4\beta_7$ integrin, and a putative mucin-like domain decorated with carbohydrate epitopes suitable for L-selectin binding [Briskin et al., 1993].

Fig. 7. Schematic illustration of the multistep model for leukocyte extravasation with focus on the orchestrated participation of selectins and integrins (for simplicity, only the neutrophil-endothelial cell interactions are shown; for details on lymphocyte homing, see text). (a) Leukocytes, freely mobile in the bloodstream, express selectin ligands and low-affinity integrins on their cell surface. (b) Initiation of the adhesion cascade. The action of inflammatory stimuli (lipopolysaccharide, N-formylated peptides, interleukin-8) released from a pathogen-infected site in the underlying tissue leads to venule dilatation, reduction in blood flow velocity and increased expression of E- and P-selectins on the surface of endothelial cells. (c) Transient adhesion events. Interactions of endothelial selectins with carbohydrate motifs (see fig. 3), displayed as oligosaccharide capping groups on membrane-associated mucin-like structures and N-linked glycans (see fig. 4), result in transient tethering and rolling of leukocytes along the endothelial surface. (d) Activation of leukocyte integrins. Probably via G-protein-linked signaling events, inflammatory cytokines can initiate triggering an increase in the affinity of leukocyte integrins towards their immunoglobulin-like ligands on the endothelial cell surface. Concomitantly, the adhesiveness of the endothelial lining for leukocytes is augmented through an enhanced expression of ICAMs. (e) Firm adhesion. Stable contacts are established by integrins of leukocytes (CD11a/CD18, CD11b/CD18), and the cells flatten in the course of this tight interaction. (f) Extravasation through endothelial cell junctions. Inset: Proposed molecular mechanism of the transmigration process with focus on platelet and endothelial cell adhesion molecule-1 (PECAM-1; CD31), a constituent of the endothelial cell intercellular junction as well as of the cell surface of blood cells such as platelets. The current status of investigation intimates that homophilic interactions between PECAM-1 molecules are crucial for leukocyte evasation with minor support by heterophilic interaction with leukocyte integrin $\alpha_v\beta_3$ [Newman, 1997].



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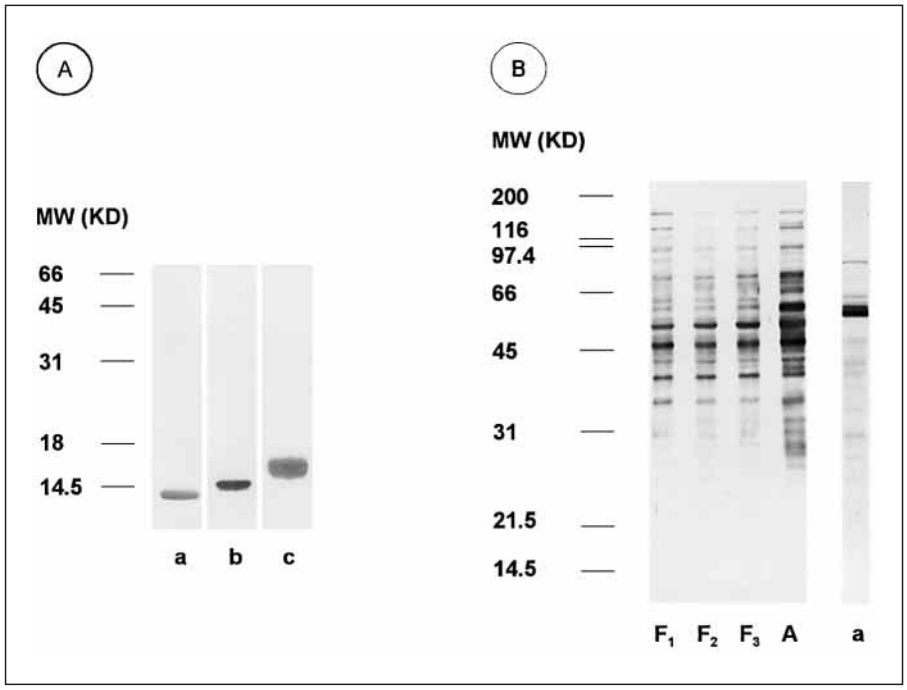


Fig. 8. A Visualization of purified bovine galectin-1 (a = MW 14,500 Da), chicken galectin from adult intestine (b = MW 14,700 Da; CG-14) and chicken galectin from adult liver (c = MW 16,000 Da; CG-16) by silver staining after reducing SDS polyacrylamide gel electrophoresis on a 15% running gel. **B** Detection of galectin-1-binding glycoproteins of extracts from bovine liver at three fetal stages (F_1 = week 7–11; F_2 = week 12–16; F_3 = week 17–21) and the adult organ (A). Protein aliquots of identical quantity (5 µg/lane) are separated by SDS polyacrylamide gel electrophoresis under reductive conditions, electroblotted onto nitrocellulose and visualized by successive incubation steps with biotinylated galectin-1 and signal-generating reagents. The effect of the presence of 0.3 *M* lactose and 0.25 mg/ml asialofetuin to block carbohydrate-dependent binding is documented for extracts from adult liver (lane a) [Kaltner et al., 1997].

the orchestrated interplay of selectins, chemoattractants, integrins and the intercellular adhesion molecules (ICAMs) makes it possible that leukocytes can transmigrate to infectious foci in underlying tissues. Molecular events in this cascade of steps are illustrated in figure 7. Following the cascade of events in an inflammatory process, selectins and their ligands cause the initial attachment and tethering of leukocytes along the vessel wall, which could be observed under intravital microscopy as 'rolling' phenomenon. Inflammatory mediators (e.g. interleukin-8) delivered from the site of infection provoke a molecular mechanism which is thought to involve via the cytoplasmic integrin domain heterotrimeric G proteins, phospholipids and protein kinases [Baggiolini, 1998]. This proposed cascade of events termed as 'inside-out' signaling results in an elevated affinity of leukocyte integrins towards their ICAM ligands on the endothelial lining. This increase appears to be due to mechanisms such as conformational changes, clustering, increase in the number of integrin molecules or changes in the interaction of integrin molecules with the cytoskeleton [Ruoslahti and Öbrink, 1996].

Figure 7 implies that a defect in integrin expression prevents firm adhesion of neutrophils to the endothelial lining and subsequent transmigration. Indeed, an inherited disorder with impaired integrin function, leukocyte adhesion deficiency I (LAD I) provides insights into the relevance of functionally active integrin molecules. In contrast to LAD II, in which the clinical symptoms are due to a defect in fucosylation, as already discussed in a preceding paragraph, LAD I is caused by heterogeneous mutations involving an initiation codon mutation or a frameshift mutation in the gene on chromosome 21q22.3 encoding CD18, the β2 leukocyte integrin subunit [Kuijpers et al., 1997]. Consequently, a deficiency of CD11/CD18 complexes on the surface of the leukocytes from LAD patients accounts for a variety of functional impairments in vitro, including abnormalities of adhesion-dependent chemotaxis, aggregation, phagocytosis of iC3b-opsonized particles, complement or antibody-dependent cytotoxicity, and transendothelial migration. The clinical symptoms of LAD I embody delayed umbilical cord severance, poor wound healing, lack of pus formation, persistent leukocytosis, soft tissue and periodontal infections, and a high risk for developing recurrent life-threatening bacterial and fungal infections. Severe and moderate phenotypes of LAD I have been described in which the gravity of clinical infections or other complications is directly related to the degree of β_2 integrin deficiency.

Although recruited leukocytes exert beneficial effects in affected sites, e.g. in wounds, serving to eliminate or con-

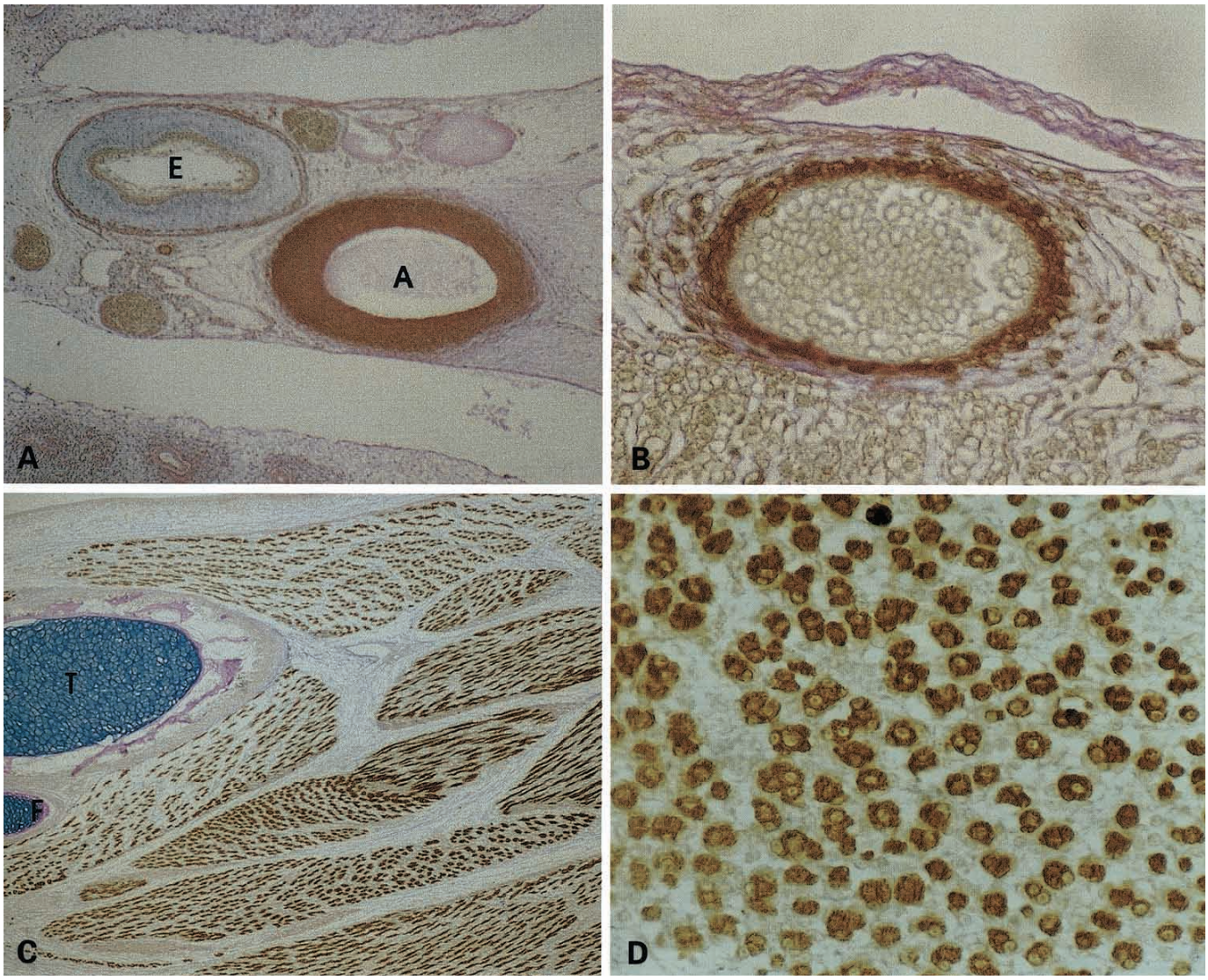
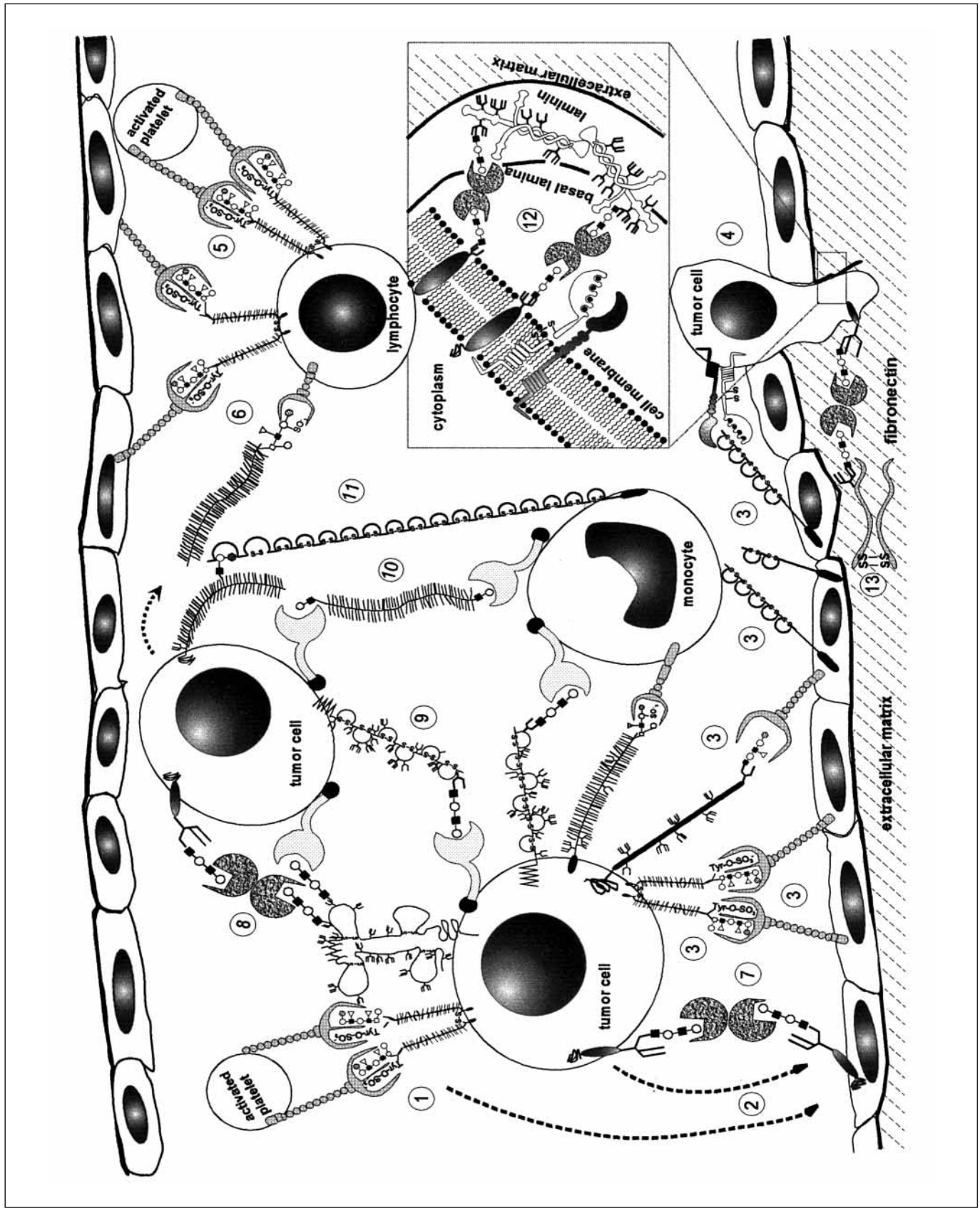


Fig. 9. Immunohistochemical localization of galectins in bovine and chicken embryonic muscle tissue. Tissue specimens were fixed in Bouin's solution, embedded in paraffin and serially cut at a thickness of 5 µm. After incubation with galectin-specific polyclonal antibodies presence of bound immunoglobulin molecules was detected by a series of incubation steps involving biotinylated anti-rabbit IgG followed by avidin-peroxidase complex and the chromogen 3, 3′-diaminobenzidine-tetrahydrochloride. All sections were briefly counterstained with the Alcian blue/periodic acid-Schiff reaction. **A** Photomicrograph of a transversal section through the thorax of a bovine embryo (55th day p.c.), incubated with affinity-purified anti-galectin-1 IgG. The aortic (A) tunica media with several layers of smooth muscle cells exhibits a strong positive reaction. The tunica muscularis of the esophagus (E) comprised of the outer longitudinal and the inner circular muscle reveals an intense staining reaction. Magnification ×55. **B** Photomicrograph of a selected part of the tissue section, presented in **A**. Distinct positive reaction of the smooth muscles of the tunica media of a vein. Magnification ×460. **C**, **D** Micrographs of a parasagittal section of striated muscle of a chick embryo on day 9 of development, incubated with affinity-purified anti-IgG against CG-16 at a magnification of ×60 (**C**) and at a magnification of ×400 (**D**).



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(For legend, see p. 175.)

trol such foci, it should be realized that an excessive migration of leukocytes can aggravate the progress of certain diseases [Albelda et al., 1994; Bevilacqua et al., 1994; Etzioni, 1996; Hillis and McLeod, 1996; for a collection of recent reviews, see Peltz, 1996]. Excessive trafficking of leukocytes to extravascular locations results in serious tissue injury and tissue destruction in both acute as well as chronic inflammatory diseases. Diverse pathological conditions including ischemia/reperfusion, organ rejection or metastasis are characterized by elevated levels of locally expressed and serum-soluble adhesion molecules. Also, various organ-specific diseases are exacerbated through exceeding extravasation of leukocytes including disorders of the cardiovascular system such as arteriosclerosis, vasculitis and thrombosis, arthritis, lung inflammation, acute and chronic CNS inflammation, hepatic, pulmonary and bowel diseases.

As discussed above, selectins recognize terminal sugar epitopes which are well-accessible. If no fucosylation is present and sialylation is incomplete, the sugar structures of N-glycans will expose galactoside-terminated structures, as shown in the chapter by Geyer and Geyer [1998] in this issue, which have become amenable by chemoenzymatic synthesis for assaying their binding affinities [André et al., 1997]. These determinants are docking sites for another class of lectins.

Galectins

The members of this lectin family share the characteristic features of Ca^{2+} independence, extensive sequence identity in the carbohydrate recognition domain and affinity for β-galactosides [Rini, 1995; Gabius, 1997a]. Galectins are widely distributed and their detection in porifera, protostomes and deuterostomes implies a common ancient ancestor of this family [Kasai and Hirabayashi, 1996]. Independent of their origin, galectins are monomeric or dimeric polypeptides with small molecular weights (14–38 kDa), as shown in figure 8A for bovine galectin-1 and two chicken galectins, namely CG-14 and CG-16. Further separation of related galectins is furnished by two-dimensional gel electrophoresis [Schneller et al., 1995]. So far, nine galectins have been reported in mammals [Gabius, 1997a]. Among the mammalian galectins, galectin-1 and galectin-3 are the best studied and occur in various tissue and cell types. Exemplarily, for histochemical studies with galectin-specific antibodies, expression of bovine galectin-1 and the chicken galectin CG-16 in smooth and skeletal muscle is shown in figure 9. With respect to tumor diagnosis, immunohistochemical galectin localization has been introduced by Gabius et al. [1986a, b] and complements the application of neoglycoconjugates in pathology [Gabius et al., 1995; Kannan and Nair, 1997; Danguy et al., 1998]. In contrast, certain galectins such as galectin-2, -4, -5 and -7 display a more restricted expression [Caron et al., 1990;

Fig. 10. Illustration of possible interactions involving endogenous lectins and appropriate glycoconjugates in the metastatic cascade. They can occur within the formation of tumor emboli, in the process of tumor cell adhesion to the endothelial lining and during the extravasation of tumor cells into the underlying tissue. Platelets can be activated by contact to tumor cells (1) resulting both in cell membrane presentation of P-selectin and in the release of platelet factors (2) which boost the expression of endothelial selectins and ICAM molecules in conjunction with tumor cell-derived factors such as TGF β or TNF α (3). In resemblance to the leukocyte-endothelial interaction cascade shown in figure 7, tumor cell-endothelial cell interaction eventually leads to extravasation of tumor cells (4). Enhanced expression of selectins (adhesion molecules) on endothelial cells provokes an increased tethering and rolling of leukocytes, which may have a bearing on homing (5). While the selectin/galectin-mediated thromboemboli formation between tumor cells and tumor cells and platelets is supposed to protect viable tumor cells from shear forces in the bloodstream, tumor cells in the aggregates are also thought to escape the surveillance mechanisms of the host immune system, advancing the transport of cancer cells to ectopic sites. Galectins (7–12) are implicated in tumor metastasis [Gabius et al., 1985b, 1994; Gabius, 1987, 1989, 1997b; Raz and Lotan, 1987; Irimura et al., 1991; Lotan et al., 1994; Ohannesian and Lotan, 1997]. Several common principles, as outlined in figure 1, are considered to participate in tumor cell aggregation and in adhesion to endothelial cells and to matrix components. Galectin-1 is present on the surface of endothelial cells (7) [Lotan et al., 1994] extending the repertoire of molecules supporting tumor cell-endothelial cell adhesion. On adjacent tumor cells the bridging mode of homodimeric galectin-1 (see fig. 1) can support tumor cell aggregation, exemplified by lysosomal membrane glycoprotein 1/2 (LAMP-1/2) [Fukuda, 1991] as ligands. Direct binding of galectin-3 to poly-N-acetyllactosamine chains of e.g. carcinoembryonic antigen (9), as known from colon carcinoma cells [Ohannesian et al., 1995], and homotypic adhesion of tumor cells probably by cross-linking of cell surface galectin-3 mediated by a soluble mucin (10) [Inohara and Raz, 1995] are further possibilities to strengthen the stability of tumor cell aggregates. I-type lectins (11) may also be involved in this complex process. It is reasonable to assume that they may recognize cancer-associated mucins on N-linked glycans owing to their content of α2,6- or α2,3-linked sialic acids in I-type lectin-reactive oligosaccharides in the peripheral part of their sugar antennae. Inset: During the metastatic process, tumor cells must cross basement membranes to invade organ parenchyma. An initial and crucial step in this process is tumor cell attachment to the basement membrane via cell surface receptors for e.g. laminin. Both galectin-1 and galectin-3 bind to N-acetyllactosamine epitopes on oligosaccharide chains on laminin (12) and fibronectin (13), justifying their definition as nonintegrin laminin/fibronectin-binding proteins, as a prerequisite for promoting spreading of tumor cells. Presence of galectins can thus modulate the adhesive properties of different tumor cell types [André et al., 1997].

Barondes et al., 1994; Kasai and Hirabayashi, 1996; Joubert-Caron et al., 1997; Gabius, 1997a; Hirabayashi, 1997; Hughes, 1997].

The recognition of specific glycoepitopes depends on the presentation of the homologous CRD which is arranged in three different types: proto-, chimera and tandem-repeat type [Kasai and Hirabayashi, 1996]. The first subgroup, the prototype, consists of polypeptides with a monomeric molecular weight of about ~14 kDa and exist under physiological conditions as noncovalent homodimers. Mammalian galectin-1, -2, -5 and -7 are categorized in this subgroup. The chimera type is represented presently by galectin-3 which is composed of a C-terminal CRD and an N-terminal accessory domain with characteristic repetitive sequences rich in proline and glycine. The typical molecular architecture of the third subgroup, the tandem-repeat type, consists of a cross-linking peptide connecting covalently two CRDs. Four members, namely, galectin-4, -6, -8 and -9 belong to this type [Gabius, 1997a].

Structural studies using different approaches such as chemical mapping procedures, determination of transferred nuclear Overhauser effects with nuclear magnetic resonance (NMR) technique, the laser photo chemically induced dynamic nuclear polarization (CIDNP) technique and X-ray analysis render it possible to dissect the interaction within a galectin-disaccharide complex on the molecular level [Rini, 1995; Siebert et al., 1996, 1997; Solís et al., 1996; von der Lieth et al., 1998]. Moving from the elucidation of molecular details of protein-carbohydrate interaction to the question of physiological relevance of lectin presence, the next step entails the description of galectin-reactive glycoligands. Consequently, methods like ligand blotting [Gabius et al., 1991a] (see also fig. 8B), immunoprecipitation, lectin affinity chromatography [Cummings, 1997] as well as histochemical colocalization with labeled tissue lectin and lectin-specific antibodies [Gabius et al., 1991a] (see fig. 9 for an experimental example) are employed to detect the galectin-reactive part of β-galactoside-containing glycoconjugates [Gabius et al., 1991b, 1993a; Gabius and Gabius, 1992, 1993; Brinck et al., 1996, 1998; Kaltner et al., 1997; Zschäbitz, 1998]. Combining histochemical galectin localization with other techniques to assess cell and tissue parameters such as growth pattern or entropy generation is assumed to be of potential value for evaluation of prognosis in tumor diagnosis [Kayser and Gabius, 1997]. Molecules fulfilling the requirements predicted for galectin ligands are for example laminin [Cooper et al., 1991], lysosome-associated membrane proteins (LAMPS-1 and LAMPS-2) [Ohannessian et al., 1994, 1995; Ohannessian and Lotan, 1997], lactosamine-containing glycolipids of olfactory neurons [Mahanthappa et al., 1994], ganglioside GM_1 [Kopitz et al., 1998], $\alpha_7\beta_1$ integrin [Gu et al., 1994], fibronectin [Ozeki et al., 1995], carcinoembryonic antigen [Ohannesian et al., 1995], colon cancer mucin [Bresalier et al., 1996] and a human brain galectin-1 ligand [Chadli et al., 1997]. The localization of these potential in vivo ligands strongly suggests that especially galectin-1 and galectin-3 are involved in adhesive interactions. Characteristically, galectins possess dual-functional roles as adhesion modulators, antagonizing or promoting adhesion. The mechanisms which are proposed for adhesive or antiadhesive interactions of galectins include direct binding of galectins to their complementary carbohydrate epitopes, 'bridging' these glycan structures on adjacent cells or ECM proteins, cross-linking of surface galectins by soluble ligands and clustering of cell surface glycans in the plasma membrane of one and the same cell via 'cis' interactions.

Galectin-1 and galectin-3 interactions with galactoside-bearing ligands seem to be integral parts in processes such as fetal development, migration of myoblasts on laminin, regulation of cell growth, tissue modeling/remodeling, apoptosis, tumor cell adhesion and metastasis. As discussed by Plendl and Sinowatz [1998] in this issue, the development of the intricate network of axonal projections in the olfactory bulb is dependent on galectin-1 [Puche et al., 1996]. Such molecular events explain the inhibition of cell adhesion by galectin-reactive sugars seen in assays such as rosette formation with certain types of tumor cells or adhesion to bone marrow stromal cells [Gabius et al., 1985a, 1990, 1993b; Raz and Lotan, 1987]. A deliberate focus on such possible roles is presented in figure 10.

Conclusion

Sugar epitopes of oligosaccharide chains linked to polypeptides or lipids contribute via recognitive interplay with specific lectins to adhesive interactions in various physiological and pathological processes. This function adds a very important chapter to the history of glycoproteins presented in this issue by Sharon [1998] with a personal touch. The elaborate status of knowledge concerning especially the lectin families of selectins and galectins verifies the involvement of carbohydrate-binding domains of these families in a series of complex steps together with other adhesion molecules such as integrins. Besides these families other adhesive molecules in the classes of C-type and I-type lectins have been unraveled, whose activities are worthy of detailed studies in the light of the presented information.

The precise understanding of the molecular details of a protein-carbohydrate interaction in an orchestrated framework of adhesion molecules is the prerequisite to define targets for the design of modulators/inhibitors of various adhesive processes. Guided by the structural elucidation of protein-carbohydrate complexes by various biophysical methods and computational modeling, as described by von der Lieth [1998] in this issue, the development of new compounds with inhibitory capacity can gain access to pharmaceuticals for therapeutic intervention in various diseases.

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