Minireview

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Leukocyte Trafficking Mediated by Selectin-Carbohydrate Interactions*

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Leukocyte emigration into lymphatic tissues or inflammatory sites is regulated by the expression of adhesion and signaling molecules (1). Binding of selectins to cell-surface carbohydrate ligands allows flowing leukocytes to attach and then roll on the vessel wall. These transient attachments expose the leukocytes to locally expressed signaling molecules. The activated leukocytes then adhere firmly through binding of activated integrins to Ig-like ligands on the endothelium. In response to chemoattractant gradients, the leukocytes also employ integrins and Ig-like molecules to migrate between endothelial cells into the tissues.

Expression of specific combinations of adhesion and signaling molecules determines the leukocyte classes that enter tissues in response to injury or immunologic challenge. However, dysregulated expression of adhesion and signaling molecules may result in thrombotic and inflammatory diseases associated with excessive leukocyte accumulation (2, 3). Here, we will focus on recent studies of the interactions of selectins with their carbohydrate ligands. Earlier reviews summarize previous work (4–7).

Physiological Expression of Selectins

Each of the three selectins has an NH_2-terminal carbohydrate-recognition domain characteristic of Ca^{2+}-dependent (C-type) lectins, followed by an epidermal growth factor (EGF)[1]-like motif, a series of short consensus repeats (SCRs), a transmembrane domain, and a cytoplasmic tail (Fig. 1). Hydrodynamic analysis and electron microscopy confirm that the selectins are rigid, asymmetric molecules (8–10).

L-selectin (CD62L), expressed on leukocytes, binds to carbohydrate ligands that are constitutively expressed on endothelial cells of peripheral lymph nodes and inducibly expressed on endothelial cells at inflammatory sites. E-selectin (CD62E) is transiently synthesized and expressed on cytokine-activated endothelial cells. P-selectin (CD62P), stored in the membranes of secretory granules in platelets and endothelial cells, is rapidly redistributed to the plasma membrane upon cellular activation by thrombin and other agonists. Both E- and P-selectin bind to carbohydrate ligands on myeloid cells and subsets of lymphocytes. Some inflammatory mediators also increase the synthesis of P-selectin (4). However, there appear to be distinct mechanisms for transcriptional regulation of E- and P-selectin, because the proteins are selectively displayed on the surface of endothelial cells at inflammatory sites (4). The cytoplasmic domain of P-selectin contains signals that mediate sorting into secretory storage granules (11), promote endocytosis from the plasma membrane (12), and enhance rapid movement from endosomes to lysosomes, where the molecule is degraded (13). Some of the P-selectin molecules that enter endosomes may recycle to the *trans*-Golgi network, where they are resorted into secretory granules (12). E-selectin on the cell surface is also rapidly internalized and digested in lysosomes (14). Thus, the levels of E- and P-selectin on the surface of endothelial cells are regulated by the balance between the rates of synthesis, storage, and degradation. Circulating forms of the selectins are generated by proteolytic cleavage or by secretion of soluble proteins encoded by alternatively spliced mRNA (4, 15). Plasma L-selectin may reach levels that partially inhibit leukocyte adhesion (16).

Studies of the regulation of expression of the selectins suggest that they mediate temporally overlapping patterns of leukocyte trafficking. These predictions are supported by *in vivo* models of leukocyte rolling or inflammation in which the functions of the selectins are probed by infusion of blocking mAbs, soluble selectins, or small carbohydrate ligands for selectins (2, 3). Furthermore, mice rendered genetically deficient in each of the selectins have clear abnormalities in leukocyte trafficking, although they develop normally. L-selectin-deficient mice have fewer lymphocytes localized to peripheral lymph nodes and are less efficient in the homing of lymphocytes to lymphatic tissues (17). Mice lacking P-selectin (18) or L-selectin (17) have defective leukocyte rolling on mesenteric venules and delayed emigration of neutrophils into the peritoneum in response to a chemical irritant. The defects in rolling occur at earlier time points in the P-selectin-deficient mice than in the L-selectin-deficient mice (19). E-selectin-deficient mice have abnormalities in leukocyte rolling and extravasation, although the defects are more obvious when P-selectin function is blocked with mAbs (20). P-selectin-deficient mice also have defective mobilization of mononuclear leukocytes in models of chronic inflammation (21, 22).

Structure-Function Studies of Selectins

The amino acid sequences of the lectin and EGF domains of the selectins are highly conserved, suggesting that both domains participate in ligand recognition. The three-dimensional structure of the lectin and EGF domains of E-selectin has been determined at 2.0-Å resolution (23); molecular modeling suggests that P- and L-selectin have domains with similar structures (24). The lectin domain of E-selectin is structurally related to the corresponding domain of rat mannose-binding protein, another C-type lectin (25). The lectin domain of E-selectin has a single Ca^{2+}-binding site located on the face opposite where the EGF domain is attached. Mutagenesis of residues on this surface impairs binding of neutrophils to E- or P-selectin (4–7, 23). Some of these residues coordinate the Ca^{2+}. A model, based on co-crystallization of an oligosaccharide with mannose-binding protein (26), suggests that sialyl Lewis x (sLe^x), a tetrasaccharide ligand bound by selectins (Fig. 2), could dock to the residues identified by mutational analysis (23). The fucose is predicted to bind to a site analogous to where mannose docks to mannose-binding protein, and the carboxylate moiety of the sialic acid is predicted to bind to adjacent, positively charged residues. However, no carbohydrate ligand has yet been co-crystallized with a selectin.

Deletion of the EGF domain impairs selectin binding to cells, consistent with its predicted importance in ligand recognition (6, 7). An L-selectin chimera containing the EGF domain of P-selectin binds endothelium of lymph nodes and, in contrast to native L-selectin, also binds leukocytes (27). It is possible that the EGF domain, rather than directly contacting a protein ligand on a target cell, affects the specificity of carbohydrate recognition by modulating the conformation of the lectin domain. However, the crystal structure of E-selectin reveals only limited contact between the lectin and EGF domains. The SCRs may also enhance ligand rec-

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[1] The abbreviations are: EGF, epidermal growth factor; ESL-1, E-selectin ligand-1; GlyCAM-1, glycosylated cell adhesion molecule-1; mAb, monoclonal antibody; MAdCAM-1, mucosal addressin cell adhesion molecule-1; PSGL-1, P-selectin glycoprotein ligand-1; SCR, short consensus repeat; sLe^x, sialyl Lewis x.

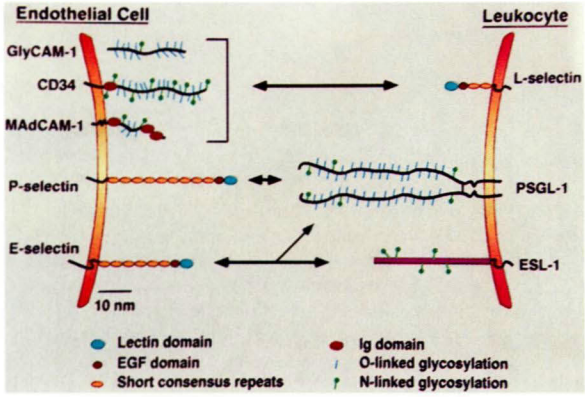

FIG. 1. **Selectins and their glycoprotein ligands.** The domain organizations of the three human selectins are shown. Their estimated lengths are based on hydrodynamic data and electron microscopy (8–10). The lengths of GlyCAM-1, CD34, MAdCAM-1, and PSGL-1 are modeled from the dimensions of another mucin-like protein, CD43 (87). The length of ESL-1 is unknown. The membrane-proximal domain of CD34 has been tentatively assigned to the Ig superfamily. GlyCAM-1 is secreted rather than attached to the membrane. PSGL-1 has been studied in humans; the other glycoprotein ligands have been studied in mice. References are as follows: P-selectin (88), E-selectin (89), L-selectin (90), GlyCAM-1 (39, 45), CD34 (39, 46), MAdCAM-1 (69, 70), and PSGL-1 (9, 36, 40, 48). Not illustrated are three less well characterized glycoproteins: a 260-kDa ligand for E-selectin (38), a 160-kDa ligand for P-selectin (52), and a 200-kDa ligand for L-selectin (91).

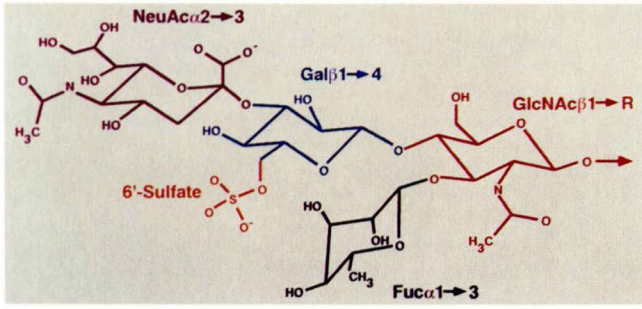

FIG. 2. **sLe^x tetrasaccharide containing a sulfate moiety at the C-6 position of the galactosyl residue.** The conformation of the oligosaccharide is derived from Refs. 44, 92, and 93.

ognition. A soluble monomer containing only the lectin and EGF domains of E-selectin is less effective in inhibiting myeloid cell adhesion to immobilized E-selectin than a monomer of E-selectin with all six SCRs (28).

Selectin Ligands

All three selectins bind sialylated and fucosylated oligosaccharides, of which the prototype is sLe^x (Fig. 2). sLe^x or related structures are linked to glycoproteins and glycolipids on most leukocytes and some endothelial cells. Leukocytes require both sialic acid and fucose on their surfaces to interact with selectins (4–7). However, the precise role of sLe^x in selectin recognition is unclear. The K_d for binding of E-selectin to sLe^x is only 0.1–1 mM (29, 30), and the affinities of L- and P-selectin for sLe^x appear to be even lower (31). E-selectin binds better to protein-associated oligosaccharides containing linear, sialylated dimeric Le^x structures (32). L- and P-selectin also bind to sulfated molecules that lack sialic acid and fucose, such as sulfatides (33) and subsets of heparin (34). The variety of carbohydrates recognized by the selectins has been reviewed (35).

The selectins bind with higher affinity or avidity to carbohydrates displayed on a limited number of glycoproteins from leukocytes or endothelial cells (Fig. 1). Most, but not all, of the glycoproteins are mucin-like, with many serine or threonine residues that are potential sites for attachment of *O*-linked glycans. Sialylation (36–39) and fucosylation (40, 41) of these glycoproteins are required for selectin recognition. Sulfation is also required for GlyCAM-1 and CD34 to bind L-selectin (42). However, the structures of the attached oligosaccharides, particularly those required for selectin recognition, are largely unknown. PSGL-1 isolated from human neutrophils has *O*-linked glycans extended with poly-*N*-acetyllactosamine, some of which terminate in sLe^x (9). GlyCAM-1 has both Gal-6-SO_4 and GlcNAc-6-SO_4 (43); Gal-6-SO_4 is attached directly to terminal sLe^x as part of the structure Siaα2–3(SO_4-6)Galβ1–4(Fucα1–3)GlcNAc (44) (Fig. 2).

A key unresolved issue is why these glycoproteins interact so well with selectins. In one model, the mucin-like glycoproteins may act as "scaffolds" for clustering of oligosaccharides (45–47). The selectin binds with low affinity to the individual glycans but with high avidity to the clustered glycans. In a variant of this model, some closely spaced glycans could create a unique recognition structure not present in any of the individual oligosaccharides (48). However, clustering of glycans is less likely to explain why E-selectin binds well to ESL-1, which has not been shown to have *O*-linked carbohydrate, and has only five sites for attachment of *N*-linked oligosaccharides on an extracellular domain of 1114 residues (41). In another model, the protein may acquire unique glycosylation or other post-translational modifications. Antibody blocking studies suggest that a specific region of PSGL-1 interacts with P-selectin (49). This region must have features that distinguish it from other *O*-glycosylated portions of the protein and from CD43, a mucin-like protein on leukocytes that does not bind P-selectin (36). Some glycosyltransferases modify only a subset of optimally presented oligosaccharides (50, 51). This mechanism might favor synthesis of minor, but critically important, oligosaccharide ligands for selectins on discrete regions of specific proteins. There are three potential sites for tyrosine sulfation near the amino terminus of PSGL-1; tyrosine sulfate displayed near an oligosaccharide(s) might create a unique binding site for P-selectin (40). Selectins may not bind equivalently to native and recombinant glycoprotein ligands, as it is not clear which glycosyltransferases or other modifying enzymes confer optimal selectin recognition.

The affinities of the selectins for the glycoprotein ligands are not known. Monomeric soluble P-selectin, when labeled with ^{125}I, binds with an apparent K_d of 70 nM to a limited number of sites on human neutrophils (8). However, the data may reflect high avidity binding of a very small percentage of oligomerized molecules, which would overestimate the affinity of monomeric P-selectin for cell-surface ligands. Binding of P-selectin to leukocytes is blocked by a mAb to PSGL-1, indicating that PSGL-1 accounts for all the high affinity/avidity binding sites for P-selectin (49). Although PSGL-1 is expressed on all lymphocytes, only a minority of these cells binds P-selectin, suggesting that the glycosylation of PSGL-1 is highly regulated (49). PSGL-1 binds to E-selectin in addition to P-selectin (9, 40, 52). Competitive binding studies indicate that both selectins recognize a related site on neutrophil-derived PSGL-1, although P-selectin binds with higher affinity to this site (9).[2] E-selectin also binds to one or more additional sites on PSGL-1.[2] Thus, one glycoprotein can be recognized by two selectins but in different ways.

Selectin-mediated Attachment and Rolling of Leukocytes under Flow

A hallmark of the selectins is their ability to mediate attachment and rolling of leukocytes on the vessel wall at shear stresses characteristic of postcapillary venules (2). This property can be studied *in vitro* by perfusing cells over a surface bearing a selectin or a selectin ligand under defined shear stresses. Neutrophils attach and roll on P- or E-selectin expressed on cell surfaces (49, 53–55)[2] or presented as purified molecules in planar membranes (56) or on plastic (57, 58).[2] Subsets of T cells also attach and roll on P- or E-selectin, perhaps through distinct ligands (59–61). L-selectin contributes to rolling of neutrophils (54) and monocytes (62) on activated endothelial cells and to rolling of neutrophils on adherent neutrophils (63).

Under flow, selectins must interact with cell-surface ligands within a fraction of a second to promote leukocyte attachment to the vessel wall. Models have been developed that describe how selectins mediate attachment and rolling of leukocytes (64, 65). The models assume that L-selectin, or ligands for P- or E-selectin, are localized on the microvilli of leukocytes, where they are likely to first contact the vessel wall. This localization has been confirmed for L-selectin (66) and PSGL-1 (49). Other modeled factors include

[2] K. D. Patel, K. L. Moore, M. U. Nollert, and R. P. McEver, submitted for publication.

clustering of selectins and their ligands to enhance binding avidity, projection of the binding domains above the glycocalyx to facilitate rapid contact, and interactions of selectins or ligands with the cytoskeleton to resist extraction from the membrane. A key prediction of the models is that the rates of unstressed bond formation and dissociation are high. Furthermore, the bond has high tensile strength; applied force does not significantly increase k_{off}. (In these analyses, a "bond" is defined as an interaction between macromolecules.) A limiting feature of the models has been the inability to independently determine the values of some parameters. This is due to uncertainty as to which selectin ligands are most important for cell adhesion, particularly under shear stresses. A glycoprotein ligand of higher affinity/avidity, although present in relatively few copies, might be essential for a cell to attach to a selectin under flow. Alternatively, larger numbers of low affinity ligands might mediate attachment under flow or stabilize adhesion after the cell first attaches.

Based on studies using different methods, it was proposed that neutrophils attach more strongly to E-selectin than to P-selectin under flow (57). However, flowing neutrophils attach more efficiently and roll with greater adhesive strength on P-selectin than on E-selectin when the selectins are compared under identical conditions.[2] A mAb to PSGL-1 blocks binding of purified PSGL-1 to P-selectin and eliminates attachment of neutrophils to P-selectin under both shear and static conditions (49). Thus, interactions between PSGL-1 and P-selectin are essential for adhesion under flow, when bond formation must occur rapidly, and in the absence of flow, when the requirements for bond formation are less stringent (Fig. 3). The same anti-PSGL-1 mAb only modestly inhibits binding of purified PSGL-1 to E-selectin and has no effect on adhesion of neutrophils to E-selectin under static conditions.[2] However, the mAb inhibits attachment of neutrophils to E-selectin under flow by ≈75%. Therefore, the region of PSGL-1 identified by the mAb also plays a role in attachment of neutrophils to E-selectin under shear stress, although other ligands for E-selectin contribute to attachment under flow and stabilize adhesion. One such ligand may be ESL-1; polyclonal antibodies to this glycoprotein partially block adhesion of neutrophils to E-selectin under static conditions (41). It is not known whether PSGL-1 mediates rolling of other leukocytes on P- or E-selectin. However, a mucin-like protein has been implicated in attachment of some T cell clones to P-selectin under flow (61).

L-selectin on neutrophils displays sLe^x and is concentrated on microvilli (66), and mAbs to L-selectin partially inhibit attachment of flowing neutrophils to E- or P-selectin (54, 55). Thus, neutrophil L-selectin may be a ligand for E- and P-selectin under flow (66). L-selectin has been suggested to be essential for the initial attachment, but not the subsequent rolling, of neutrophils on E-selectin (58). However, human HL-60 cells lack L-selectin, yet attach and roll on both P- and E-selectin in a PSGL-1-dependent manner (49).[2] *In vivo*, HL-60 cells also roll on venules when P-selectin is expressed on the endothelial surface (67). Perhaps L-selectin interacts with PSGL-1 on the same neutrophil, clustering both molecules on the microvilli. If so, mAbs to L-selectin might indirectly inhibit the function of PSGL-1 by reducing its oligomerization in the membrane.

In an attempt to examine the kinetics of selectin-ligand bonds, neutrophils were perfused over a planar membrane containing P-selectin at densities below those required to support rolling (68). Transient attachments were observed with apparent first-order kinetics, which were interpreted as unimolecular interactions between P-selectin and PSGL-1. A plot was made of the number *versus* the duration of attachments to yield a cellular detachment rate, which increased only modestly with increasing shear stress. The results support models that selectin-ligand bonds require fast on and off rates plus a high tensile strength to mediate rolling under physiological shear stress (64, 65). However, it was assumed that single bonds were being measured and that P-selectin and PSGL-1 were resistant to removal from lipid bilayers. Biochemical measurements of the unstressed on and off rates of binding of PSGL-1 to P-selectin are required to validate the cellular experiments.

Of the described glycoprotein ligands for selectins, only PSGL-1 has been documented to mediate cell-cell interactions under flow (49).[2] L-selectin mediates attachment of flowing lymphocytes to MAdCAM-1 coated on glass (69). On intact endothelial cells, however, the oligosaccharides on MAdCAM-1 may not project above the glycocalyx to allow rapid contacts with L-selectin (70). Lymphoid tissues secrete GlyCAM-1 into plasma, where it may competitively inhibit L-selectin-dependent leukocyte adhesion (71). It will be essential to identify the principal ligand(s) on each cell type that interacts with each selectin under shear stress. It should be noted that selectins are not uniquely capable of mediating attachment and rolling of leukocytes under shear forces. The $\alpha4\beta1$ and $\alpha4\beta7$ integrins mediate attachment, rolling, and firm adhesion of mononuclear cells and eosinophils to Ig-like ligands (60, 72–74).

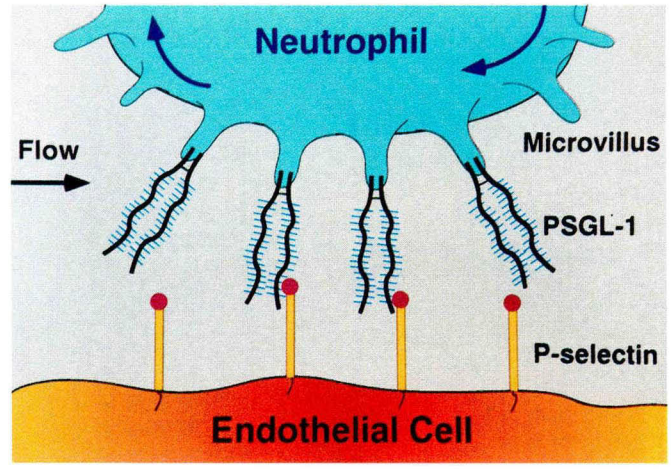

FIG. 3. **Interactions of PSGL-1 with P-selectin mediate attachment and rolling of neutrophils under flow.** Rapid contacts between the two molecules should be favored by the projection of their binding domains above the glycocalyx and by the clustering of PSGL-1 on the tips of microvilli. It has not been determined whether other identified glycoprotein ligands participate in selectin-mediated attachment and rolling of leukocytes under shear stress.

Signaling through Selectins and Modulation of Selectin Function by Signaling

Leukocytes roll at velocities that are lower than those of free-flowing cells (1), allowing juxtacrine activation by locally expressed signaling molecules (75). Direct signaling through selectin-ligand interactions might also occur, although this remains an unsettled issue. There are reports that binding of P-selectin signals neutrophils (76–78) and monocytes (79). In other studies, however, P-selectin does not detectably activate neutrophils or monocytes, but it enhances the effects of agonists such as platelet-activating factor (75, 80, 81). Purified E-selectin reportedly stimulates neutrophils (82), but neutrophils attached to E-selectin are not obviously activated (54, 57, 58).[2] The priming or signaling of adherent leukocytes may be regulated by the density or clustering of selectins and their ligands. For example, antibody cross-linking of L-selectin signals neutrophils (83).

Upon activation of leukocytes, L-selectin function is transiently enhanced (84), perhaps through cytoskeletally dependent mechanisms (85). L-selectin is then proteolytically shed from the cells (86). Activated neutrophils adhere less avidly to P-selectin, even in the absence of flow.[3] The diminished adhesiveness is not due to removal of PSGL-1 from the cell surface but is associated with a cytoskeletally dependent redistribution of PSGL-1-dependent binding sites for P-selectin. Loss of L-selectin and redistribution of PSGL-1 may facilitate transfer of adhesive control from selectins to integrins as the activated leukocyte begins to emigrate into the tissues.

Summary

The selectins have attracted intense interest because of their carbohydrate-recognition properties and their pivotal roles in leukocyte trafficking. Future studies will center on the mechanisms for regulating the expression of the selectins and their ligands, the molecular details of selectin binding to glycoprotein ligands and

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small carbohydrates, and the biophysical principles that selectins employ to mediate attachment and rolling of leukocytes under flow.

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