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Selectin-carbohydrate interactions during inflammation and metastasis

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L-, E-, and P-selectin are membrane-anchored, C-type lectins that initiate tethering and rolling of flowing leukocytes on endothelial cells, platelets, or other leukocytes during inflammation. The selectins bind to sialylated, fucosylated, or, in some cases, sulfated glycans on glycoproteins, glycolipids, or proteoglycans. However, they bind with relatively high affinity or avidity to only a few, appropriately modified glycoproteins on leukocytes or endothelial cells. One leukocyte mucin, PSGL-1, tethers flowing leukocytes to P-selectin on activated platelets or endothelial cells, and also helps tether leukocytes to L-selectin on other leukocytes. The physiologic expression of the selectins is tightly controlled to limit the inflammatory response. But dysregulated expression of the selectins may contribute to inflammatory and thrombotic disorders, and perhaps to tumor metastases.

***Keywords*: C-type lectins, inflammation, metastasis, mucin, leukocytes, platelets, endothelial cells**

Introduction

The selectins are a family of three membrane-anchored Ca^{2+}-dependent (C-type) lectins that bind to cell-surface carbohydrate ligands. These interactions promote adhesion of leukocytes to platelets, endothelial cells, or other leukocytes in response to infection or tissue injury. Selectin-ligand bonds form rapidly but transiently, allowing a free-flowing leukocyte to tether to and then roll on the vessel wall under the shear forces characteristic of postcapillary venules. The rolling leukocytes encounter regionally presented chemokines or lipid autacoids that activate the leukocytes. Interactions of leukocyte integrins with immunoglobulin-like counter-receptors strengthen adhesion and direct emigration into the underlying tissues in response to chemotactic gradients. Expression of distinct combinations of adhesion and signaling molecules controls the duration and specificity of leukocyte recruitment [1, 2]. This review focuses on aspects of selectin-ligand recognition, selectin-dependent leukocyte attachment under shear forces, physiologic regulation of selectin or their ligands in inflammation, and pathologic expression of selectins in disease states. Earlier reviews provide additional information and references [3–5].

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Selectin-ligand recognition

Each selectin has an amino-terminal carbohydrate-recognition domain characteristic of C-type lectins, followed by an epidermal growth factor (EGF)-like module, a series of short consensus repeats (SCRs), a transmembrane domain, and a short cytoplasmic tail (Figure 1). L-selectin, expressed on most leukocytes, binds to constitutively expressed ligands on high endothelial venules (HEV) of peripheral lymph nodes, to inducible ligands on endothelium at sites of inflammation, and to ligands on other leukocytes. E-selectin, expressed on activated endothelial cells, and P-selectin, expressed on activated platelets and endothelial cells, bind to ligands on myeloid cells and subsets of lymphocytes. P-selectin may also bind to ligands on HEV or on activated endothelial cells.

Like all C-type lectins, the selectins bind to carbohydrate ligands in a Ca^{2+}-dependent manner. The three-dimensional structure of the lectin and EGF domains of E-selectin has been solved by X-ray crystallography [6]. Site-directed mutagenesis of selectins, interpreted in the context of the E-selectin structure, suggests that carbohydrate binds to the lectin domain on a shallow region that overlaps a single Ca^{2+} coordination site opposite where the EGF domain is attached [6–11]. Several studies with selectin constructs in which the EGF domains and/or SCRs have been deleted, switched, or mutated suggest that these domains contribute to ligand specificity [12–15]. However, one group finds no obvious difference in ligand specificity when the EGF

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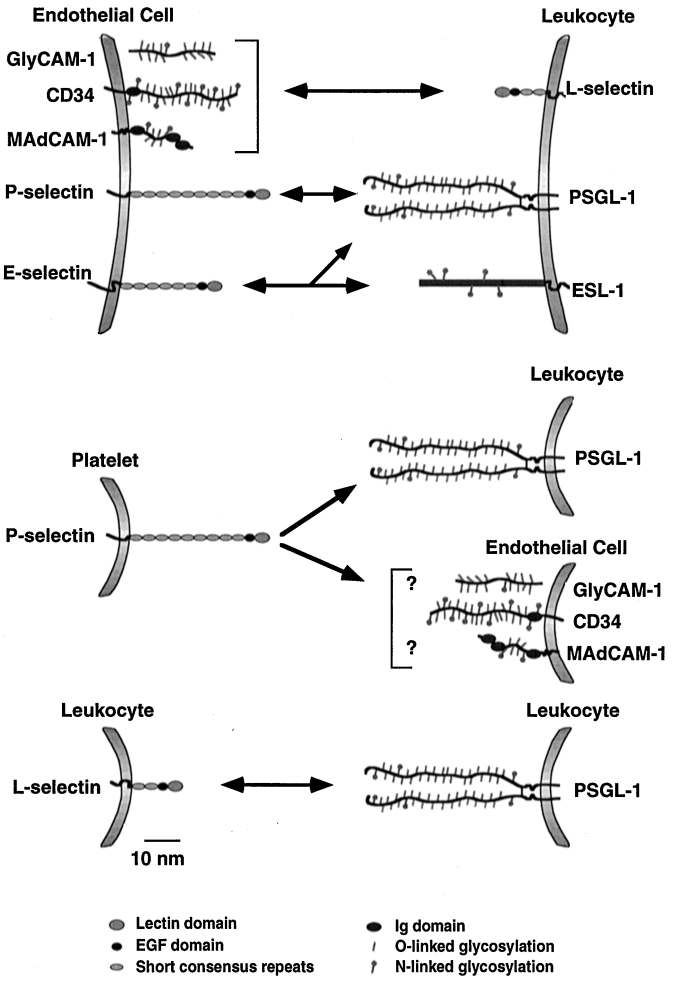

Figure 1. Selectins and their glycoprotein ligands. The estimated lengths of the selectins [17, 128, 129] and of PSGL-1 [51] are based on hydrodynamic data and electron microscopy. The lengths of GlyCAM-1, CD34 and MAdCAM-1 are modeled from the dimensions of another sialomucin, CD43 [130]. Not shown are less well-characterized selectin ligands: a 260 kDa bovine leukocyte ligand for E-selectin [131], a 160 kDa murine leukocyte ligand for P-selectin [132], a 200 kDa murine endothelial cell ligand for L-selectin [25] and CD24, a leukocyte ligand for P-selectin [133]. Abbreviations: ESL-1, E-selectin ligand-1; GlyCAM-1, glycosylated cell adhesion molecule-1; MAdCAM-1, mucosal addressin cell adhesion molecule-1; PSGL-1, P-selectin glycoprotein ligand-1.

domains and SCRs of E- and L-selectin are exchanged [16]. The selectins are linear molecules with little obvious contact between the various domains [6, 17], and it remains unclear how the EGF domains and SCRs might affect the binding function of the lectin domain.

The selectins bind sialylated and fucosylated oligosaccharides such as sialyl Lewis x (sLe^x; Neu5Acα2,3Galβ1,4 [Fucα1,3]GlcNAc-R), a terminal component of glycans attached to glycoproteins and glycolipids on most leukocytes and some endothelial cells [18, 19]. Although these cells must be sialylated and fucosylated to interact with selectins, the affinity of selectins for isolated sLe^x-related oligosaccharides is very low. Furthermore, L- and P-selectin, but not E-selectin, bind sulfated molecules such as heparin and sulfatides [18, 19]. Clearly the selectins bind with higher affinity to only a few glycoproteins on leukocytes or endothelial cells (Figure 1). These glycoproteins must be sialylated and fucosylated to interact with the selectins [20–23]. Furthermore, they must be sulfated to bind optimally to L- or P-selectin [24–29]. Studies of these glycoproteins have focused on the specific nature of the post-translational modifications that confer high affinity binding to selectins, and the potential functions of these glycoproteins in mediating selectin-dependent cell adhesion under hydrodynamic flow.

Most of the described glycoprotein ligands are sialomucins that bind better to L- and/or P-selectin, although they do bind to E-selectin under some conditions. Binding requires sialylated and fucosylated O-glycans; the limited number of N-glycans on these molecules appears to be dispensable for binding. The fucosylated glycans on GlyCAM-1 from murine HEV are short core-2 structures that lack polylactosamine [30–32]. In contrast, most fucosylated O-glycans on PSGL-1 from human myeloid cells are core-2 structures that have a β1,6-linked trifucosylated polylactosamine terminating in sLe^x [33]. Only 14% of the O-glycans of PSGL-1 are fucosylated [33]. These data suggest that unique O-glycan structures are created at restricted sites on specific proteins. Sulfate esters are attached to the C-6 position of Gal and GlcNAc residues in the O-glycans of GlyCAM-1 [30–32]. In contrast, the O-glycans of PSGL-1 are not sulfated [33]. Instead, sulfate is attached to a group of three clustered tyrosines near the amino terminus [26–29]. High affinity binding of P- or L-selectin to GlyCAM-1 may require appropriate spacing of two or more sialylated, fucosylated O-glycans. High affinity binding to PSGL-1 may require appropriate presentation of one or more tyrosine sulfates located near one or more amino-terminal, sialylated and fucosylated, O-glycans. Selectins may bind to specific composite recognition sites on other sialomucins, including some derived from malignant cells [34].

E-selectin, but not P- or L-selectin, binds to ESL-1, a glycoprotein on leukocytes with up to five N-glycans but no demonstrated O-glycans [21, 22]. The structures of the N-glycans have not been elucidated.

Selectin-mediated tethering and rolling of leukocytes under hydrodynamic flow

Many *in vitro* and *in vivo* studies have demonstrated that leukocytes use selectins to tether to and roll on the vessel wall under the shear forces characteristic of postcapillary venules (reviewed in refs [1–3, 5]). Leukocytes roll on the endothelium through interactions of L-selectin with constitutively or inducibly expressed ligands on the endothelial

cell surface, and through interactions of E- and P-selectin on the activated endothelium with ligands on the leukocytes. Leukocytes roll on P-selectin expressed by activated adherent platelets. Leukocytes also use L-selectin to roll on adherent leukocytes [35, 36] or to initiate leukocyte aggregates [37]. Leukocyte-leukocyte and leukocyte-platelet interactions may be a major mechanism for amplifying the recruitment of leukocytes to the endothelial cell surface under shear forces [38–41].

Under flow, selectin-ligand interactions must form rapidly to facilitate tethering, and then dissociate rapidly to facilitate rolling. Furthermore, shear forces must not significantly accelerate the rate of dissociation [42]. L-selectin-ligand interactions have faster rates of association and dissociation than P- or E-selectin-ligand interactions. Leukocytes interacting through L-selectin require a threshold shear force to support rolling, because faster rotation is required to bring L-selectin molecules at the leading edge in proximity to new ligands on the substrate before the bonds at the trailing edge of the cell dissociate [43]. Other factors also regulate the efficiency of selectin-dependent tethering and rolling under flow conditions. Cell-cell contact may induce proteolytic shedding of L-selectin, accelerating rolling velocity by reducing the number of effective L-selectin-ligand bonds [44]. L-selectin is clustered on the tips of microvilli, which markedly enhances its ability to contact ligands on a surface under shear forces [45]. Flowing neutrophils attach and roll much less effectively on transfected cells expressing P-selectin molecules that are shortened by deletion of some of the SCRs [46].

Of the described glycoprotein ligands for selectins, PSGL-1 has the most clearly demonstrated function in tethering and rolling of leukocytes under flow conditions. PL1, a mAb to human PSGL-1, prevents binding of purified PSGL-1 to purified P-selectin [47]. The mAb also blocks tethering and rolling of leukocytes on P-selectin substrates *in vitro* [47, 48] and *in vivo* [49]. Flowing leukocytes also tether to and roll on purified PSGL-1; this interaction is blocked by PL1 and by mAbs to L-selectin [38]. Furthermore, PL1 significantly inhibits the L-selectin-dependent rolling of neutrophils on adherent neutrophils [38] and the L-selectin-dependent aggregation of stirred neutrophils [50]. There are, however, L-selectin ligands other than PSGL-1 that participate in leukocyte-leukocyte contacts [39, 40]. Under some conditions, PL1 inhibits the accumulation of rolling neutrophils on E-selectin [48], but this effect may be indirect through inhibition of L-selectin-PSGL-1 interactions between leukocytes [38, 50]. The PL1 epitope is near the amino terminus of PSGL-1; it overlaps the tyrosine sulfation sites and includes a threonine to which a critical O-glycan may be attached [51]. This supports the concept that P- and L-selectin bind to a composite, amino-terminal recognition site. Like P-selectin, PSGL-1 is a highly extended molecule, which projects its binding site well above the cell surface [51]. Like L-selectin, PSGL-1 is concentrated on the tips of microvilli [47]. Thus, the structure and orientation of PSGL-1 are ideally suited for efficient interactions with P- and L-selectin under flow.

Physiologic expression of selectins and their ligands

The expression of selectins is normally tightly regulated to ensure that leukocytes tether to and roll on the blood vessel wall only at appropriate locations. L-selectin is proteolytically shed from leukocytes after cell-cell contact and after cellular activation, a mechanism that modulates and then downregulates its function [44]. E- and P-selectin are only expressed on the surface on endothelial cells and/or platelets after the cells are activated. Tumor necrosis factor α (TNF-α), interleukin-1β, or lipopolysaccharide (LPS) transiently induce endothelial cells to transcribe E-selectin mRNA, which leads to synthesis of E-selectin protein. Surface expression of E-selectin peaks within 4 h after activation and then usually declines over the course of 12–24 h [3–5]. In contrast, P-selectin is constitutively synthesized by megakaryocytes and endothelial cells, where it is stored in the α granules of platelets and the Weibel-Palade bodies of endothelial cells. Mediators such as thrombin, histamine, complement components, and oxygen-derived radicals cause rapid redistribution of P-selectin to the cell surface through fusion of granule membranes with the plasma membrane [3–5]. In mice, TNF-α or LPS also increase P-selectin mRNA and protein levels, with kinetics similar to those observed for the induction of E-selectin [52–54]. These mediators do not augment P-selectin mRNA levels in human endothelial cells, suggesting that there may be species-specific mechanisms for regulation of P-selectin gene expression [55, 56]. However, the cytokines IL-4 or oncostatin M markedly augment P-selectin mRNA levels in cultured human endothelial cells in a delayed and sustained fashion [56].

P-selectin remains on the surface of activated platelets for at least 1 h, which may stabilize platelet-leukocyte conjugates [57]. On the other hand, P- and E-selectin are rapidly internalized from the surface of activated endothelial cells [58, 59]. P-selectin is endocytosed through clathrin-coated pits [60]. Although both P- and E-selectin recycle from early endosomes to the plasma membrane, they have short half-lives because they are rapidly degraded in lysosomes [59, 61–63]. The short half-life of P-selectin is due to efficient sorting from endosomes to lysosomes [61]. Some P-selectin molecules may also recycle from endosomes to the trans-Golgi network where they enter new Weibel-Palade bodies [64]. The 35-residue cytoplasmic domain of P-selectin contains signals that direct sorting into secretory granules [65], endocytosis in clathrin-coated pits [60], and movement from endosomes to lysosomes [61]. A fourth signal may direct sorting into synaptic-like vesicles [66], although this compartment is not likely to be significant in endothelial cells. The steady-state distribution of P- and

E-selectin in endothelial cells reflects the balance between their rates of synthesis and their rates of sorting into various subcellular compartments. For example, human endothelial cells treated with IL-4 express some P-selectin on the cell surface, presumably because the increased synthesis saturates the sorting pathway from the trans-Golgi network to secretory granules and diverts some newly synthesized P-selectin to the plasma membrane. Subsequent stimulation with histamine further increases surface P-selectin during fusion of Weibel-Palade bodies with the plasma membrane [56].

The physiologic functions of the selectins *in vivo* have been confirmed in many animal models in which blocking mAbs to selectins were employed (reviewed in refs. [67, 68]). The importance of selectins in humans is underscored by the discovery of a congenital disorder of fucose metabolism, termed leukocyte adhesion deficiency 2 (LAD-2) [69–71]. Because patients with LAD-2 lack fucosylated glyconjugates, they do not express functional selectin ligands on leukocytes or, presumably, on endothelial cells. Leukocytes from these patients do not tether to and roll on P- or E-selectin surfaces. Clinically, the patients have more infectious diseases, supporting the concept that the selectins have an important function in initiating recruitment of leukocytes.

Mice made genetically deficient in each of the three selectins appear healthy, but they have obvious defects in leukocyte trafficking in response to specific challenges [54, 72, 73]. Lymphocytes from L-selectin-deficient mice home less efficiently to peripheral lymph nodes [73]. Mice lacking L- or P-selectin demonstrate impaired rolling of leukocytes in venules of exteriorized mesentery [54, 73]. The defect in rolling is observed earlier after tissue exteriorization in P-selectin-deficient mice, consistent with the rapid mobilization of P-selectin to the endothelial cell surface after trauma [74]. Comparison of the kinetics and degree of leukocyte rolling among wild-type, L-selectin-deficient, and P-selectin-deficient mice suggest that L- and P-selectin function cooperatively during acute inflammatory responses [68, 74]. This may reflect the overlapping expression of both P-selectin and an L-selectin ligand on the endothelial cell surface [75]. In addition, L-selectin-dependent leukocyte-leukocyte contacts and P-selectin-dependent platelet-leukocyte contacts may significantly amplify the number of flowing cells that attach to the vessel wall [38–41, 50]. Mice deficient in either L- or P-selectin have impaired leukocyte recruitment in models of acute and chronic inflammation [54, 76, 77]. Such defects are less obvious in E-selectin-deficient mice, but can be elicited by blocking P-selectin function by infusion of a mAb [72], Mice lacking both E- and P-selectin have frequent severe infections and shortened survival [78, 79]. It is not obvious why these mice have more infections than do LAD-2 patients, whose lack of fucosylated ligands should lead to defective recognition by all three selectins. The phenotypic differences emphasize that the expression or functions of selectins or their ligands may not be identical in mice and humans.

In vitro, P- and L-selectin bind to human hematopoietic progenitor cells, most likely through PSGL-1 [80–84]. *In vitro* studies also suggest that E-selectin contributes to angiogenesis [85, 86]. However, neither selectin-deficient mice nor LAD-2 patients have defects in hematopoiesis other than myelopoiesis associated with infections. Furthermore, neither the mice nor the patients have obvious abnormalities in angiogenesis or wound healing. It is possible that selectins normally participate in these processes, but that other molecules substitute in their absence.

Pathologic expression of selectins

Excessive accumulation of leukocytes contributes to the pathogenesis of inflammatory disorders such as ischemia-reperfusion injury, Gram-negative shock, and rheumatoid arthritis [87, 88]. Tissue injury results from release of oxygen-derived radicals, proteases, and other mediators. Dysregulated expression of selectins has been implicated in several forms of leukocyte-mediated tissue injury.

Activated complement and oxygen radicals, which are frequently present during the early stages of sepsis or ischemia-reperfusion syndromes, mobilize P-selectin to the surface of endothelial cells *in vitro* [89–92]. Oxygen radicals prolong the expression of P-selectin on the cell surface, perhaps by inhibiting endocytosis [90]. Endothelial dysfunction decreases formation of nitric oxide, an oxygen-radical scavenger that may normally dampen the expression of P-selectin [93]. Hypoxia also translocates P-selectin to the surface of endothelial cells [94, 95]. Consistent with these observations, ischemia-reperfusion induces expression of P-selectin on endothelial cells *in vivo* [96–98]. Furthermore, mAbs and other P-selectin inhibitors significantly reduce neutrophil accumulation and tissue injury in many ischemia-reperfusion models [96, 99–106]. Antibodies to P-selectin decrease neutrophil accumulation in the tissues of rats injected with LPS [107] and in some models of acute lung injury in rats [108]. Antibodies to L-selectin also reduce tissue injury in models of ischemia-reperfusion [109, 110] and in some models of acute lung injury [111]. Thus, just as L- and P-selectin function cooperatively in physiologic leukocyte recruitment, they may cooperatively enhance acute leukocyte-mediated tissue injury in some disease states. Selectins have been less well studied in models of chronic inflammatory disease. However, their contributions to pathogenesis may be inferred from their observed expression on venular endothelial cells from patients with some chronic or allergic inflammatory disorders [112–114].

Oxidized low-density lipoprotein activates both platelets and endothelial cells, promoting P-selectin-dependent platelet-leukocyte aggregates and leukocyte adhesion to the arterial endothelium *in vitro* and *in vivo* [115]. Cigarette smoke, which causes release of oxygen radicals, produces

similar effects *in vivo* [116]. Some viral infections prolong the expression of P-selectin on the surface of cultured endothelial cells [117]. These insults may allow monocytes and other leukocytes to emigrate beneath the endothelium during the early stages of atherosclerosis. In the later stages of atherosclerosis, P-selectin is observed on the apical surface of the endothelium [114], perhaps because of the local synthesis of IL-4 or oncostatin M by subendothelial macrophages or T cells [56]. The endothelially expressed P-selectin may promote recruitment of additional monocytes, particularly in areas of arterial bifurcation where shear stresses are lower. Rupture of advanced atherosclerotic plaques promotes platelet aggregation and thrombin formation. Leukocytes accumulating on adherent platelets may express tissue factor, further augmenting thrombin and fibrin generation [118]. Consistent with this notion, mAbs to P-selectin accelerate pharmacological thrombolysis in a primate model of arterial thrombosis [119]. Anti-P-selectin mAbs also reduce infiltration of inflammatory cells in a rat model of venous thrombosis [120].

These data suggest that inhibitors of selectin function or expression might be effective therapeutics in some inflammatory or thrombotic disorders. A potential risk of such agents is interference with the physiologic recruitment of leukocytes required to combat infections. However, antibodies to P- or L-selectin do not significantly increase infections in some experimental models, suggesting that other adhesion molecules may suffice for this purpose [121, 122]. Furthermore, infections are relatively uncommon in LAD-2 patients [69]. The limited number of infections may result from the ability of $\alpha 4$ integrins to tether flowing mononuclear leukocytes and eosinophils to the endothelium [123, 124].

Some cancer cells may metastasize by employing selectins or selectin ligands normally used for leukocyte or platelet adhesion, although this hypothesis has not been directly tested in animal models of metastasis. All three selectins bind to some malignant cells or cell lines [34, 125, 126]. Many of the ligands on these tumor cells appear to be sialomucins. Some malignant leukemia or lymphoma cells express L-selectin [127]. Tumor cells might attach to P-selectin on both activated endothelial cells and platelets, forming multicellular aggregates on the surface of the microvasculature.

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