Perspectives series: cell adhesion in vascular biology. Role of PSGL-1 binding to selectins in leukocyte recruitment.

R P McEver, R D Cummings

J Clin Invest. 1997;100(3):485-491. https://doi.org/10.1172/JCI119556.

Perspective

Perspectives Series: Cell Adhesion in Vascular Biology

Role of PSGL-1 Binding to Selectins in Leukocyte Recruitment

Rodger P. McEver*‡§‖ and Richard D. Cummings‡

**Department of Medicine and ‡Department of Biochemistry and Molecular Biology, and the §W.K. Warren Medical Research Institute, University of Oklahoma Health Sciences Center, and the ‖Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104*

A multistep series of adhesive and signaling events regulates inflammatory responses to infection or injury (1–3). To initiate these responses, circulating leukocytes must adhere to the vascular wall under shear forces. Selectins mediate the first adhesive step, which is characterized by tethering and rolling of leukocytes on endothelial cells, platelets, or other leukocytes (4, 5). L-selectin, expressed on most leukocytes, binds to ligands on some endothelial cells and on other leukocytes. E-selectin, expressed on cytokine-activated endothelial cells, binds to ligands on most leukocytes. P-selectin, expressed on activated platelets and endothelial cells, also binds to ligands on most leukocytes. The regulated expression of the selectins and their ligands helps initiate and terminate the inflammatory response. However, inappropriate expression of these molecules contributes to leukocyte-mediated tissue damage in a variety of inflammatory and thrombotic disorders (6).

Each selectin is a type 1 membrane glycoprotein with an NH_2-terminal C-type lectin domain, followed by an EGF-like domain, a series of short consensus repeats, a transmembrane domain, and a short cytoplasmic tail. Selectins mediate cell–cell adhesion through interactions of the lectin domains with specific glycoconjugate ligands. Like other mammalian lectins, the selectins bind selectively, but with low affinity, to particular oligosaccharides. All selectins bind to the tetrasaccharide sialyl Lewis x (sLe^x;[1] NeuAcα2,3Galβ1,4[Fucα1,3]GlcNAc) and its isomer sialyl Lewis a (sLe^a; NeuAcα2,3Galβ1,3[Fucα1,4]GlcNAc). L- and P-selectins, but not E-selectin, also bind to particular sulfated carbohydrates, such as heparan sulfate, that lack sialic acid and fucose (4, 5, 7). However, selectins bind with higher affinity or avidity to only a few glycoproteins. Most of these are mucins, i.e., glycoproteins with multiple Ser/Thr-linked oligosaccharides (O-glycans) and repeating peptide motifs (4, 5). A key issue is whether any of these molecules mediates biologically relevant interactions with selectins (7). This perspective focuses on P-selectin glycoprotein ligand-1 (PSGL-1), a sialomucin with the most clearly defined function as a selectin ligand.

Identification of a specific glycoprotein ligand for P-selectin on leukocytes

The original observation that P-selectin binds preferentially to a single glycoprotein in human myeloid cells was revealed by ligand blotting and affinity chromatography experiments (8). The glycoprotein, now termed PSGL-1, was found to be a disulfide-bonded homodimer with two 120-kD subunits as determined by SDS-PAGE under reducing and nonreducing conditions (Fig. 1). Digestion with peptide *N*-glycosidase F demonstrated that PSGL-1 has at most two or three N-glycans that are not required for binding to P-selectin (8). However, experiments with sialidases indicated that α2,3-linked sialic acid is required for P-selectin binding, suggesting that PSGL-1 expresses functional sialylated O-glycans. Indeed, the glycoprotein was found to contain the sLe^x antigen and to have many sialylated, clustered O-glycans that render it susceptible to cleavage with *O*-sialoglycoprotein endopeptidase (9). Treatment of intact myeloid cells with *O*-sialoglycoprotein endopeptidase eliminates the high-affinity binding sites for P-selectin without affecting overall surface expression of sLe^x (9, 10). These data suggested that PSGL-1 is the major ligand for P-selectin on intact myeloid cells as well as in cell lysates.

Primary structure of PSGL-1

A cDNA encoding PSGL-1 was subsequently isolated from a human HL-60 cell library by expression cloning using COS cells that were panned on immobilized P-selectin (11). Functional expression of PSGL-1 in COS cells required cotransfection with an α1,3 fucosyltransferase, confirming earlier observations that both α1,3 fucosylation and α2,3 sialylation of surface glycoproteins are required for binding to P-selectin (12). The deduced amino acid sequence of PSGL-1 predicts a type 1 membrane protein of 402 amino acids (Fig. 1). It has an NH_2-terminal signal peptide, followed by a propeptide that is cleaved by paired basic amino acid converting enzymes. The extracellular domain of the mature protein, which begins at residue 42, has the hallmarks of a mucin. It is rich in serines, threonines, and prolines, and includes 15 decameric repeats. Three NH_2-terminal tyrosines at residues 46, 48, and 51 are located in an anionic consensus sequence that favors tyrosine sulfation. There is a single extracellular cysteine located at the

Address correspondence to Rodger P. McEver, M.D., W.K. Warren Medical Research Institute, University of Oklahoma Health Sciences Center, 825 N.E. 13th Street, Oklahoma City, OK 73104. Phone: 405-271-6480; FAX: 405-271-3137; E-mail: rodger-mcever@ouhsc.edu

Received for publication 18 June 1997 and accepted in revised form 23 June 1997.

1. *Abbreviations used in this paper:* CHO, Chinese hamster ovary; C2GnT, core-2 β1,6 *N*-acetylglucosaminyltransferase; PSGL-1, P-selectin glycoprotein ligand-1; sLe^x, sialyl Lewis x.

J. Clin. Invest.

0021-9738/97/08/0485/08 $2.00
Volume 100, Number 3, August 1997, 485–492
http://www.jci.org

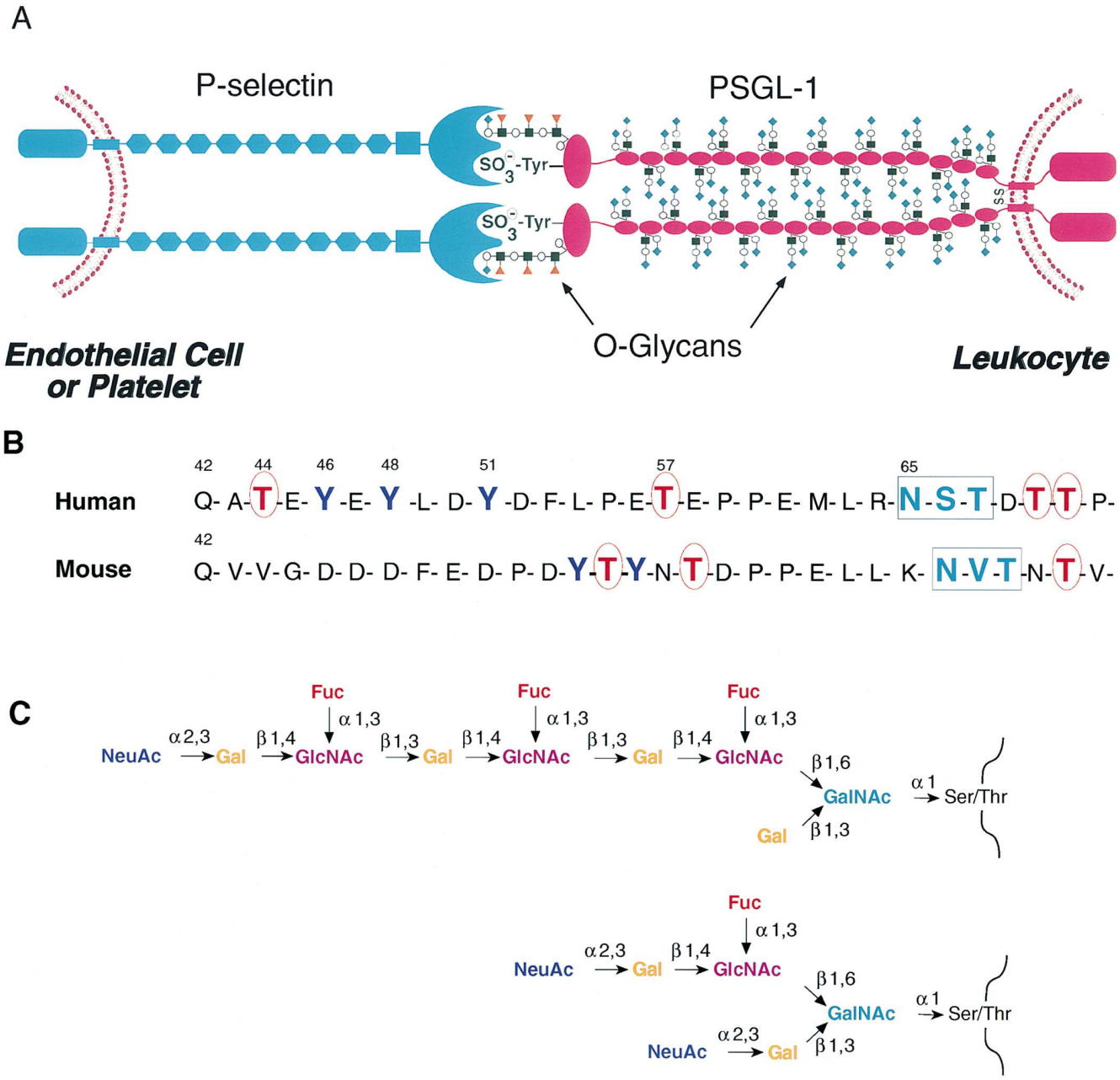

Figure 1. (*A*) Model for interactions between P-selectin and PSGL-1. A P-selectin molecule is depicted binding to each of the two subunits of PSGL-1. The C-type lectin domain of P-selectin makes contacts with an NH_2-terminal sLe^x-containing, core-2 O-glycan and a nearby tyrosine sulfate residue of PSGL-1. Also shown are the EGF domain and the nine short consensus repeats in the extracellular domain of P-selectin, and the 16 decameric consensus repeats in the extracellular domain of PSGL-1. The symbols on the O-glycans of PSGL-1 represent sialic acid (*filled diamonds*), galactose (*open circles*), *N*-acetylglucosamine (*filled boxes*), and fucose (*filled triangles*). (*B*) NH_2-terminal amino acid sequences of mature human and murine PSGL-1. The threonines that are potential sites for O-glycosylation are circled. The consensus sites for N-glycosylation are enclosed by rectangles. The tyrosines located in consensus regions for tyrosine sulfation are marked in boldface. The numbering system is based on the full-length precursor proteins, each of which has an NH_2-terminal signal peptide and propeptide that are cleaved after synthesis. (*C*) Structures of the two fucosylated, core-2 O-glycans on PSGL-1 derived from HL-60 cells.

junction of the transmembrane domain, which is followed by a cytoplasmic domain of 69 residues. The cDNA for murine PSGL-1 reveals a protein of similar size to the human protein. Murine PSGL-1 also has a signal peptide, a propeptide, and a single cysteine near the transmembrane domain (13). Furthermore, murine PSGL-1 has an anionic NH_2-terminal sequence with two rather than three tyrosines (Fig. 1). The sequences of the murine and human transmembrane and cytoplasmic domains are highly conserved, implying important functions. The murine extracellular domain, although rich in serines, threonines, and prolines, has only 10 decameric repeats, and shares little sequence similarity with the human protein. A single

exon encodes the open reading frame in both the human and murine PSGL-1 genes (13, 14). Interestingly, the sequence of PSGL-1 in most human leukocytes has an additional decameric repeat not found in the protein from HL-60 cells and other cell lines (14, 15).

Posttranslational modifications of PSGL-1 required for binding to P-selectin

As discussed above, PSGL-1 must be modified with α2,3-linked sialic acid and α1,3-linked fucose to bind P-selectin (8, 9, 11). These modifications must be on O-glycans, because enzymatic removal of N-glycans or mutations that prevent N-glycan addition do not inhibit binding (8) (Epperson, T.K., R.P. McEver, and R.D. Cummings, unpublished observations). Endoglycosidase treatments indicated that PSGL-1 from human neutrophils has O-glycans with sialylated and fucosylated polylactosamines $[\rightarrow 3Gal\beta1\rightarrow 4GlcNAc\beta1\rightarrow]_n$ (16). Extension with polylactosamine commonly occurs only on the β1,6 branch of core-2 O-glycans (17). The importance of specific O-glycans for expression of functional PSGL-1 was revealed by studying recombinant forms of the glycoprotein in Chinese hamster ovary (CHO) cells. CHO cells synthesize only simple core-1 O-glycans because they lack the core-2 β1,6 *N*-acetylglucosaminyltransferase (C2GnT) that creates the core-2 structure (17, 18). To generate glycoforms of PSGL-1 that bind with high affinity to P-selectin, CHO cells must be cotransfected with cDNAs encoding both an α1,3 fucosyltransferase and C2GnT (19). Thus, PSGL-1 requires α2,3 sialylated and α1,3 fucosylated, core-2 O-glycans to interact with P-selectin.

The structures of the O-glycans on PSGL-1 from human HL-60 cells have been determined (20). Remarkably, only 14% of the O-glycans are fucosylated, which occur as two distinct core-2 species (Fig. 1). The more abundant species is a novel structure with a sialylated, trifucosylated polylactosamine extension on the β1,6 branch. Myeloid cells express two α1,3 fucosyltransferases, FTIV and FTVII (21–23). The relative contributions of these two enzymes for construction of the fucosylated O-glycans of PSGL-1 are not known. Unlike the O-glycans on PSGL-1, the O-glycans on CD43, a sialomucin of similar size expressed on HL-60 cells, have virtually no fucose and almost no polylactosamine (17, 20). Both PSGL-1 and CD43 have many short core-2 O-glycans, indicating that formation of the core-2 branch is not sufficient for polylactosamine extension or fucosylation. These data suggest that fucosylated O-glycans are constructed only at specific sites on specific proteins. The paucity of fucosylated O-glycans on PSGL-1 indicates that the protein is not merely a scaffold for attaching many clustered sLe^x-bearing O-glycans that increase the avidity of binding to P-selectin. Consistent with this notion, Fab fragments of the anti–PSGL-1 mAb PL1 block binding of P-selectin to purified PSGL-1 and to intact leukocytes (15). PL1 identifies an NH_2-terminal protein epitope spanning residues 49–62 of PSGL-1, suggesting that it masks a single preferred binding site for P-selectin on or near this region (24). In contrast, mAb PL2, which identifies a protein epitope in the decameric consensus repeats (24), does not block binding of PSGL-1 to P-selectin (15).

Human PSGL-1 is also sulfated (25–27), but the sulfate is present exclusively on tyrosine residues rather than on O-glycans (20, 25). Sulfation occurs on one or more of the three clustered tyrosines at residues 46, 48, and 51 (26, 27). Enzymatic removal of sulfate (25), blockade of sulfate synthesis (26, 27), proteolytic removal of an NH_2-terminal fragment containing the three clustered tyrosines (28), or replacement of the tyrosines with phenylalanines (19, 26, 27) eliminates binding of PSGL-1 to P-selectin. When coexpressed in COS cells with an α1,3 fucosyltransferase, an Ig chimera containing only residues 42–60 of PSGL-1 binds to P-selectin, although perhaps not as well as the full-length molecule (27). Together, these data suggest that P-selectin binds preferentially to an NH_2-terminal region of PSGL-1 that includes at least one tyrosine sulfate and at least one sialylated and fucosylated, core-2 O-glycan.

The structural basis for the requirement for both specific O-glycosylation and tyrosine sulfation of PSGL-1 to confer binding to P-selectin is unknown. In human PSGL-1, Thr-44 and Thr-57 are the potential attachment sites for O-glycans that are closest to the clustered tyrosines (Fig. 1). Alanine substitution of both residues diminishes binding of truncated or chimeric forms of recombinant PSGL-1 to P-selectin (26, 27). In the context of full-length recombinant PSGL-1, alanine substitution of Thr-57 eliminates binding to P-selectin, whereas substitution of Thr-57 with serine, to which an O-glycan might also be attached, does not prevent binding. Furthermore, alanine substitution of Thr-44 or of residues on either side of Thr-57 has no effect on binding (Liu, W., J. Kang, R.D. Cummings, and R.P. McEver, manuscript submitted for publication). These data strongly suggest that P-selectin binding requires a specific O-glycan attached to a threonine at residue 57 (residue 16 of the mature, proteolytically processed protein). Specific biochemical characterization is required to determine whether either of the fucosylated O-glycans shown in Fig. 1 is attached to this residue. Little is known about the sequence requirements that favor construction of specific O-glycans on mucins. Therefore, the putative O-glycan attached to residue 57 might not be identical in full-length PSGL-1 or in a chimera containing only an NH_2-terminal fragment of PSGL-1.

The stoichiometry of sulfation of native PSGL-1 is not known. Human full-length recombinant PSGL-1 coexpressed with C2GnT and an α1,3 fucosyltransferase in CHO cells still binds P-selectin when any two of the three tyrosines are replaced with phenylalanines (Liu, W., J. Kang, R.D. Cummings, and R.P. McEver, manuscript submitted for publication). This suggests that each of the three tyrosines is sulfated under some conditions, and that only one tyrosine sulfate is required for binding. Whether additional tyrosine sulfates increase binding affinity remains to be determined. Murine PSGL-1 has two threonines at residues 55 and 58 and two tyrosines at residues 54 and 56 (Fig. 1). Polyclonal and monoclonal antibodies raised to an NH_2-terminal peptide spanning residues 42–60 of murine PSGL-1 block binding of murine PSGL-1 to P-selectin (13, 29, 30). Binding of P-selectin to this region of murine PSGL-1 suggests some permissible variation in the number of tyrosine sulfates and their proximity in the amino acid sequence to the attachment site for the required O-glycan.

Other structural features of PSGL-1 may also be important for optimal binding to P-selectin. The acidic residues surrounding the tyrosines may favor binding, although they are not sufficient in the absence of tyrosine sulfate. Dimerization of PSGL-1 may increase binding avidity by simply presenting two recognition determinants on a single molecule. But dimerization could also increase binding affinity, for example, by placing tyrosine sulfate on one polypeptide chain in a favorable orientation with an O-glycan on the other chain. Three-dimensional structures determined from cocrystallization of

P-selectin with an appropriate fragment of PSGL-1 are ultimately needed to identify the molecular contacts.

Posttranslational modifications of PSGL-1 required for binding to L- and E-selectins

PSGL-1 has also been shown to bind to both L-selectin (31–33) and E-selectin (11, 16, 34, 35). Thus, the term PSGL-1 is misleading because the mucin is not a glycoprotein ligand for only P-selectin. Binding of PSGL-1 to L-selectin is blocked by mAb PL1 (31–33, 36), by enzymatic removal of the NH_2-terminal clustered tyrosines (33), or by the prevention of sulfate synthesis (33). These results suggest that L- and P-selectins bind to a similar NH_2-terminal region of PSGL-1 that requires both tyrosine sulfate and O-glycan(s). However, it remains possible that L-selectin recognizes a different combination of tyrosine sulfate and O-glycan(s) than does P-selectin.

PSGL-1 binds much differently to E-selectin than to P- or L-selectin. Core-2, sialylated and fucosylated O-glycans are required for binding to E-selectin (19), but tyrosine sulfation is not required (19, 26, 27). E-selectin also binds to the NH_2-terminal region of PSGL-1 (16, 37), although with lower affinity than does P-selectin (16). The anti–PSGL-1 mAb PL1 has little or no effect on binding of PSGL-1 to E-selectin. This and other data indicate that E-selectin also binds to one or more still uncharacterized sites on PSGL-1 (37, 38). The relative binding affinities of PSGL-1 for P-, L-, and E-selectins are not known. It is also not known whether a specific, fucosylated O-glycan structure (Fig. 1) favors binding to a particular selectin.

Tissue distribution of PSGL-1

Northern blot analysis indicated that mRNA for PSGL-1 is expressed in many human and murine organs, but the analysis did not indicate the specific cells in which it is expressed (11, 13). Flow cytometric and immunocytochemical analysis of multiple human tissues with the anti–PSGL-1 mAbs PL1 or PL2 revealed that the PSGL-1 core protein is expressed primarily in hematopoietic cells (15, 39). In bone marrow it is expressed on myeloid cells at many stages of maturation, but not on erythroid cells, megakaryocytes, or platelets. PSGL-1 is expressed on virtually all leukocytes, but at lower levels on B cells. P-selectin binds to PSGL-1 on all myeloid cells (15). However, it binds to PSGL-1 on only a subset of T cells (15, 40); most of these are memory cells (41) and they may be predominantly γ/δ cells (42). This suggests that differentiating T cells alter the posttranslational modifications of PSGL-1 in order to confer P-selectin recognition. Consistent with this hypothesis, levels of C2GnT and α1,3 fucosyltransferase activity rise in T cells after antigen stimulation in vitro (40). PSGL-1 is expressed on circulating dendritic cells, on tissue monocyte-derived dendritic cells, and on some dendritic cells in lymphoid organs; the function of the protein in these cells is unknown. PSGL-1 is also expressed on some $CD34^+$ stem cells (39), where it may bind P-selectin (43). This interaction might modulate stem cell homing or hematopoietic cell differentiation.

The PSGL-1 protein is also expressed on epithelial cells lining the fallopian tube and in some endothelial cells at sites of chronic inflammation (39). It has been reported that P-selectin is present on the surface of porcine sperm, where it binds to a P-selectin ligand, which may be related to PSGL-1, on the zona pellucida of porcine oocytes (44). The physiological significance of this phenomenon has not been determined.

Function of PSGL-1–selectin interactions in tethering and rolling of leukocytes under hydrodynamic flow

Under hydrodynamic flow, selectin–ligand interactions must form rapidly to facilitate tethering, and then dissociate rapidly to facilitate rolling. Furthermore, selectin–ligand bonds must have mechanical strength so that shear forces do not significantly accelerate the rate of dissociation (2). There are relatively few copies of PSGL-1 on leukocytes (10, 15), and PSGL-1

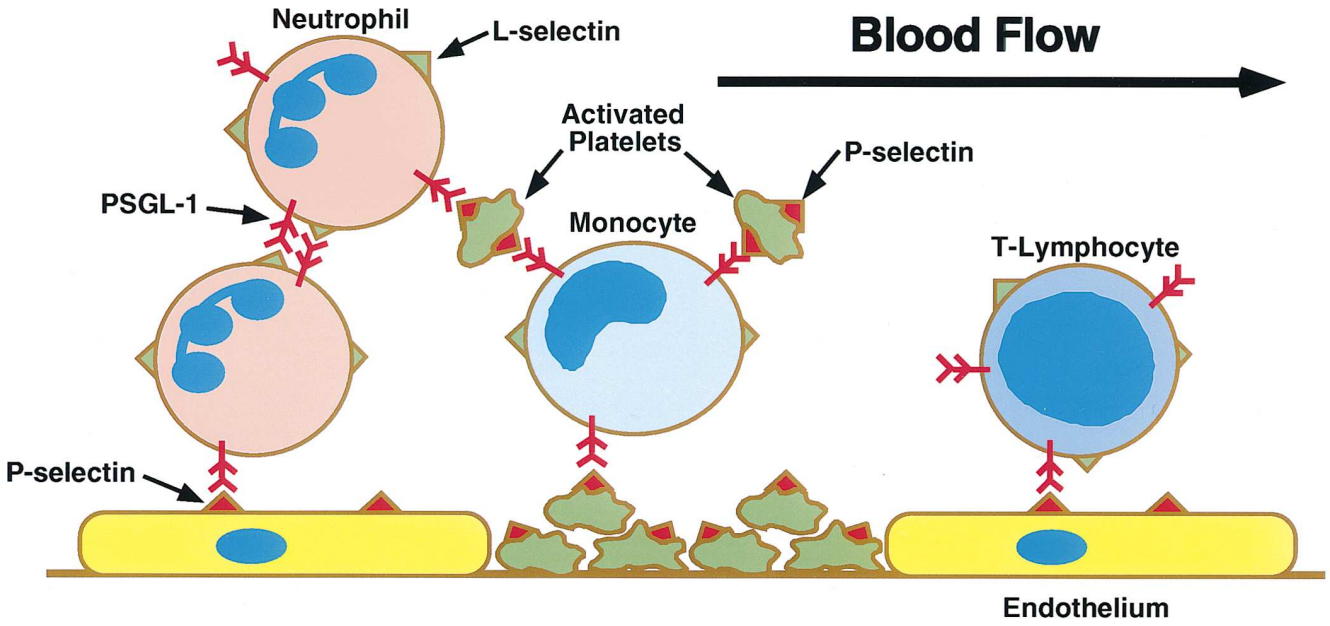

Figure 2. Multicellular interactions mediated by binding of PSGL-1 to P- and L-selectins under hydrodynamic flow. Binding of PSGL-1 to P-selectin promotes tethering and rolling of leukocytes on activated endothelial cells and platelets. Binding of PSGL-1 to L-selectin mediates tethering of leukocytes to other leukocytes, which may amplify recruitment of leukocytes to the vascular wall. Activated platelets, through P-selectin–PSGL-1 interactions, may connect additional leukocytes to sites of inflammation or tissue injury.

displays only a small fraction of the total sLe^x-containing glycans on the cell surface (9). However, PSGL-1 is the only high-affinity ligand for P-selectin on intact leukocytes (15), and PSGL-1 is the essential ligand for mediating adhesion of leukocytes to P-selectin. The anti–PSGL-1 mAb PL1 blocks tethering and rolling of human neutrophils, eosinophils, and mononuclear cells on P-selectin under flow (15) (Patel, K.D., and R.P. McEver, unpublished data). PL1 also abrogates adhesion of neutrophils and monocytes to P-selectin under static conditions (15, 45, 46). More recently, an mAb to an NH_2-terminal epitope of murine PSGL-1 has been shown to block tethering and rolling of murine myeloid cells on P-selectin under flow (30). Thus, interactions of PSGL-1 with P-selectin mediate adhesion of leukocytes to both activated endothelial cells and platelets under shear stress (Fig. 2).

The requirement for PSGL-1 to mediate leukocyte adhesion to P-selectin probably reflects, in part, its superior binding affinity relative to other ligands. The orientations of both PSGL-1 and P-selectin on the cell surface may also optimize their interactions under hydrodynamic flow. Both P-selectin and PSGL-1 are highly extended proteins, which project their NH_2-terminal binding domains above most of the cell surface glycocalyx (10, 24). Indeed, most of the O-glycans on PSGL-1 may function primarily to extend the NH_2-terminal region above the plasma membrane. When expressed on transfected CHO cells, shortened P-selectin constructs with fewer short consensus repeats are much less effective than wild-type P-selectin in mediating tethering and rolling of neutrophils under flow (47). PSGL-1 is also concentrated on microvillous tips (15, 48). Thus, both the lengths and surface distributions of PSGL-1 and P-selectin may enhance rapid and specific interactions, yet minimize nonspecific repulsion between apposing cell surfaces. Dimerization of PSGL-1 may increase the k_{on} and/or decrease the k_{off} of binding to P-selectin, as may clustering of dimers on microvillous tips. Biochemical evidence suggests that P-selectin may also form oligomers (10). Therefore, dimers or clusters of both PSGL-1 and P-selectin may regulate the rates of bond formation and dissociation under flow conditions. Upon neutrophil activation, PSGL-1 undergoes a cytoskeletal-dependent redistribution to the uropods of polarized cells (48–50). This redistribution is associated with weakening of adhesion to P-selectin and transfer of adhesive control to integrins (49, 50).

Like PSGL-1, L-selectin is also concentrated on the tips of microvilli (51). Leukocytes use L-selectin to roll on adherent leukocytes (52) or to initiate leukocyte aggregation (53). Leukocyte–leukocyte interactions lead to secondary tethering of leukocytes to an P- or E-selectin surface, a potential mechanism for amplifying leukocyte recruitment to the vessel wall under shear forces (31, 54). Flowing leukocytes roll on purified PSGL-1; this interaction is blocked by PL1 and by mAbs to L-selectin (31). Furthermore, PL1 significantly inhibits the L-selectin–dependent rolling of neutrophils on adherent neutrophils (31) and the L-selectin–dependent aggregation of stirred neutrophils (36). These data suggest that PSGL-1 is an important ligand for L-selectin under at least some conditions (Fig. 2). However, there are L-selectin ligands other than PSGL-1 that participate in leukocyte–leukocyte contacts (31, 54–56).

PSGL-1 may be one of only a few glycoproteins in human leukocyte lysates that binds well to E-selectin (34, 57). Microspheres coated with recombinant PSGL-1 also roll on immobilized E-selectin under shear forces (37). However, whether PSGL-1 has any significant function for adhesion of leukocytes to E-selectin remains to be demonstrated. PL1 partially reduces accumulation of rolling neutrophils on E-selectin under flow (38). But this effect occurs indirectly through inhibition of L-selectin–PSGL-1 interactions between neutrophils, thus reducing secondary tethering of neutrophils to E-selectin (Patel, K.D., and R.P. McEver, manuscript submitted for publication). PL1 blocks primary tethering of flowing leukocytes to P-selectin but not to E-selectin (54) (Patel, K.D., and R.P. McEver, manuscript submitted for publication). Human K562 cells transfected with FTVII roll on E-selectin in the absence of PSGL-1 (58). Conversely, eosinophils, which express PSGL-1 but express relatively little total sLe^x, tether and roll much less efficiently on E-selectin than on P-selectin (59) (Patel, K.D., and R.P. McEver, manuscript submitted for publication). Together, these data suggest that E-selectin must bind to ligands other than PSGL-1 to mediate leukocyte attachment under flow. These observations underscore the premise that favorable receptor–ligand interactions in solution need not predict favorable adhesive interactions between cells under shear stress.

Signaling through PSGL-1

In the multistep model of leukocyte recruitment, leukocytes rolling on endothelial cells or platelets encounter regionally presented chemokines and lipid autacoids that stimulate the leukocytes to develop integrin-dependent firm adhesion and other responses. However, signals may also be directly transmitted through adhesion molecules (1). The available data suggest that binding of P-selectin to PSGL-1 on leukocytes generates signals that must be integrated with those from other activators to elicit most effector responses (1). In the best studied examples, monocytes mobilize the transcription factor NF-κB and synthesize the cytokines TNF-α and monocyte chemotactic protein-1 when the cells adhere to immobilized P-selectin and platelet-activating factor, but not to either molecule alone (45). Monocytes secrete a different profile of cytokines when they are exposed to P-selectin and the platelet-derived chemokine, RANTES, but not to either protein alone (46). Under some conditions, cooperative signaling through PSGL-1 and receptors for conventional activators may also generate other leukocyte responses (1, 60).

The nature of the signals transmitted through PSGL-1 requires further study. Adhesion of T cells to P-selectin was reported to induce tyrosine phosphorylation of the pp^{125} focal adhesion kinase (FAK), although the role of PSGL-1 in this event was not directly tested (61). pp^{125}FAK has not been detected in human myeloid cells (62). However, engagement of PSGL-1 with bivalent mAbs or immobilized P-selectin induces rapid tyrosine phosphorylation of other proteins in human neutrophils (Hidari, K.I.-J., A.S. Weyrich, G.A. Zimmerman, and R.P. McEver, manuscript submitted for publication). These include the ERK family of mitogen-activated protein kinases, which are activated by PSGL-1 engagement. Engagement of PSGL-1 with mAbs is sufficient to stimulate neutrophils to secrete IL-8. This secretion is blocked by a tyrosine kinase inhibitor, suggesting that tyrosine phosphorylation propagated through PSGL-1 may be physiologically important (Hidari, K.I.-J., A.S. Weyrich, G.A. Zimmerman, and R.P. McEver, manuscript submitted for publication). Cross-linking of L-selectin also rapidly transmits signals into both myeloid

and lymphoid cells (63–68). Thus, binding of L-selectin to PSGL-1 during leukocyte–leukocyte interactions may transmit bidirectional, potentially cooperative, signals during the earliest phases of leukocyte recruitment.

Physiological and pathological functions of PSGL-1–selectin interactions

Recent in vivo studies have confirmed the predictions from in vitro experiments that PSGL-1 is a physiologically important selectin ligand. Anti–PSGL-1 mAbs inhibit rolling of both human and murine leukocytes on P-selectin expressed in postcapillary venules in vivo (30, 69). Polyclonal antibodies directed to the NH_2-terminal segment of murine PSGL-1 specifically inhibit the recruitment of T helper 1 lymphocytes in a delayed-type hypersensitivity model (29). An mAb to the NH_2-terminal region of murine PSGL-1 also inhibits accumulation of neutrophils into chemically inflamed peritoneum (30). The degree of inhibition is comparable to that observed with an mAb to P-selectin. More complete inhibition is observed with the combined use of mAbs to both PSGL-1 and P-selectin, suggesting that PSGL-1 interacts with at least one other molecule. An obvious candidate is L-selectin, given the in vitro evidence that binding of L-selectin to PSGL-1 mediates leukocyte–leukocyte interactions.

Since PSGL-1 promotes adhesive interactions through both P- and L-selectins, it is almost certain to contribute to pathological leukocyte recruitment in a variety of inflammatory and thrombotic disorders in which P- and L-selectins have been implicated previously (6). This suggests that mAbs to PSGL-1, soluble forms of PSGL-1, and other inhibitors of PSGL-1 function are potentially useful antiinflammatory drugs in such conditions. In support of this concept, infusion of recombinant soluble PSGL-1 potently inhibits leukocyte infiltration and parenchymal damage in rat kidneys subjected to ischemia and reperfusion. Proteolytic removal of the NH_2-terminal region of PSGL-1 abrogates its protective effects (70). This implies that soluble PSGL-1 blocks adhesion of leukocytes to P-selectin and perhaps to L-selectin in this model. In other pathological states, soluble PSGL-1 may also be an effective E-selectin inhibitor even if PSGL-1 on leukocyte surfaces is not a dominant ligand for E-selectin.

Conclusions

The binding of selectins to PSGL-1 is a prototype for a biologically relevant lectin–mucin interaction that mediates cell adhesion and signaling. Many interesting questions remain to be answered. These include defining the details of the posttranslational modifications that confer optimal PSGL-1 binding to selectins, the nature of the molecular contacts between lectin and ligand, the biophysical parameters that facilitate cell–cell interactions under flow, the molecular mechanisms for PSGL-1–mediated leukocyte signaling, and the role of PSGL-1–selectin interactions in vivo during physiological and pathological inflammation, hemostasis, and hematopoiesis.

References

1. Zimmerman, G.A., T.M. McIntyre, and S.M. Prescott. 1996. Adhesion and signaling in vascular cell–cell interactions. *J. Clin. Invest.* 98:1699–1702.

2. Konstantopoulos, K., and L.V. McIntire. 1997. Effects of fluid dynamic forces on vascular cell adhesion. *J. Clin. Invest.* 98:2661–2665.

3. Springer, T.A. 1995. Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. *Annu. Rev. Physiol.* 57:827–872.

4. McEver, R.P., K.L. Moore, and R.D. Cummings. 1995. Leukocyte trafficking mediated by selectin-carbohydrate interactions. *J. Biol. Chem.* 270: 11025–11028.

5. Kansas, G.S. 1996. Selectins and their ligands: current concepts and controversies. *Blood.* 88:3259–3287.

6. Lowe, J.B., and P.A. Ward. 1997. Therapeutic inhibition of carbohydrate–protein interactions in vivo. *J. Clin. Invest.* 99:822–826.

7. Varki, A. 1997. Selectin ligands: will the real ones please stand up? *J. Clin. Invest.* 99:158–162.

8. Moore, K.L., N.L. Stults, S. Diaz, D.L. Smith, R.D. Cummings, A. Varki, and R.P. McEver. 1992. Identification of a specific glycoprotein ligand for P-selectin (CD62) on myeloid cells. *J. Cell Biol.* 118:445–456.

9. Norgard, K.E., K.L. Moore, S. Diaz, N.L. Stults, S. Ushiyama, R.P. McEver, R.D. Cummings, and A. Varki. 1993. Characterization of a specific ligand for P-selectin on myeloid cells. A minor glycoprotein with sialylated *O*-linked oligosaccharides. *J. Biol. Chem.* 268:12764–12774.

10. Ushiyama, S., T.M. Laue, K.L. Moore, H.P. Erickson, and R.P. McEver. 1993. Structural and functional characterization of monomeric soluble P-selectin and comparison with membrane P-selectin. *J. Biol. Chem.* 268:15229–15237.

11. Sako, D., X.-J. Chang, K.M. Barone, G. Vachino, H.M. White, G. Shaw, G.M. Veldman, K.M. Bean, T.J. Ahern, B. Furie, et al. 1993. Expression cloning of a functional glycoprotein ligand for P-selectin. *Cell.* 75:1179–1186.

12. Zhou, Q., K.L. Moore, D.F. Smith, A. Varki, R.P. McEver, and R.D. Cummings. 1991. The selectin GMP-140 binds to sialylated, fucosylated lactosaminoglycans on both myeloid and nonmyeloid cells. *J. Cell Biol.* 115:557–564.

13. Yang, J., J. Galipeau, C.A. Kozak, B.C. Furie, and B. Furie. 1996. Mouse P-selectin glycoprotein ligand-1: molecular cloning, chromosomal localization, and expression of a functional P-selectin receptor. *Blood.* 87:4176–4186.

14. Veldman, G.M., K.M. Bean, D.A. Cumming, R.L. Eddy, S.N.J. Sait, and T.B. Shows. 1995. Genomic organization and chromosomal localization of the gene encoding human P-selectin glycoprotein ligand. *J. Biol. Chem.* 270:16470–16475.

15. Moore, K.L., K.D. Patel, R.E. Bruehl, L. Fugang, D.A. Johnson, H.S. Lichenstein, R.D. Cummings, D.F. Bainton, and R.P. McEver. 1995. P-selectin glycoprotein ligand-1 mediates rolling of human neutrophils on P-selectin. *J. Cell Biol.* 128:661–671.

16. Moore, K.L., S.F. Eaton, D.E. Lyons, H.S. Lichenstein, R.D. Cummings, and R.P. McEver. 1994. The P-selectin glycoprotein ligand from human neutrophils displays sialylated, fucosylated, *O*-linked poly-*N*-acetyllactosamine. *J. Biol. Chem.* 269:23318–23327.

17. Maemura, K., and M. Fukuda. 1992. Poly-*N*-acetyllactosaminyl *O*-glycans attached to leukosialin: the presence of sialyl Le^x structures in *O*-glycans. *J. Biol. Chem.* 267:24379–24386.

18. Sasaki, H., B. Bothner, A. Dell, and M. Fukuda. 1987. Carbohydrate structure of erythropoietin expressed in Chinese hamster ovary cells by a human erythropoietin cDNA. *J. Biol. Chem.* 262:12059–12076.

19. Li, F., P.P. Wilkins, S. Crawley, J. Weinstein, R.D. Cummings, and R.P. McEver. 1996. Post-translational modifications of recombinant P-selectin glycoprotein ligand-1 required for binding to P- and E-selectin. *J. Biol. Chem.* 271: 3255–3264.

20. Wilkins, P.P., R.P. McEver, and R.D. Cummings. 1996. Structures of the O-glycans on P-selectin glycoprotein ligand-1 from HL-60 cells. *J. Biol. Chem.* 271:18732–18742.

21. Lowe, J.B., J.F. Kukowska-Latallo, R.P. Nair, R.D. Larsen, R.M. Marks, B.A. Macher, R.J. Kelly, and L.K. Ernst. 1991. Molecular cloning of a human fucosyltransferase gene that determines expression of the Lewis x and VIM-2 epitopes but not ELAM-1-dependent cell adhesion. *J. Biol. Chem.* 266: 17467–17477.

22. Natsuka, S., K.M. Gersten, K. Zenitas, R. Kannagi, and J.B. Lowe. 1994. Molecular cloning of a cDNA encoding a novel human leukocyte α1,3-fucosyltransferase capable of synthesizing the sialyl Lewis x determinant. *J. Biol. Chem.* 269:16789–16794.

23. Maly, P., A.D. Thall, B. Petryniak, G.E. Rogers, P.L. Smith, R.M. Marks, R.J. Kelly, K.M. Gersten, G.Y. Cheng, T.L. Saunders, et al. 1996. The α(1,3)Fucosyltransferase Fuc-TVII controls leukocyte trafficking through an essential role in L-, E-, and P-selectin ligand biosynthesis. *Cell.* 86:643–653.

24. Li, F., H.P. Erickson, J.A. James, K.L. Moore, R.D. Cummings, and R.P. McEver. 1996. Visualization of P-selectin glycoprotein ligand-1 as a highly extended molecule and mapping of protein epitopes for monoclonal antibodies. *J. Biol. Chem.* 271:6342–6348.

25. Wilkins, P.P., K.L. Moore, R.P. McEver, and R.D. Cummings. 1995. Tyrosine sulfation of P-selectin glycoprotein ligand-1 is required for high affinity binding to P-selectin. *J. Biol. Chem.* 270:22677–22680.

26. Pouyani, T., and B. Seed. 1995. PSGL-1 recognition of P-selectin is controlled by a tyrosine sulfation consensus at the PSGL-1 amino terminus. *Cell.* 83:333–343.

27. Sako, D., K.M. Comess, K.M. Barone, R.T. Camphausen, D.A. Cumming, and G.D. Shaw. 1995. A sulfated peptide segment at the amino terminus of PSGL-1 is critical for P-selectin binding. *Cell.* 83:323–331.

28. De Luca, M., L.C. Dunlop, R.K. Andrews, J.V. Flannery, Jr., R. Ettling, D.A. Cumming, G.M. Veldman, and M.C. Berndt. 1995. A novel cobra venom metalloproteinase, mocarhagin, cleaves a 10-amino acid peptide from the ma-

ture *n* terminus of P-selectin glycoprotein ligand receptor, PSGL-1, and abolishes P-selectin binding. *J. Biol. Chem.* 270:26734–26737.

29. Borges, E., W. Tietz, M. Steegmaier, T. Moll, R. Hallmann, A. Hamann, and D. Vestweber. 1997. P-selectin glycoprotein ligand-1 (PSGL-1) on T helper 1 but not on T helper 2 cells binds to P-selectin and supports migration into inflamed skin. *J. Exp. Med.* 185:573–578.

30. Borges, E., R. Eytner, T. Moll, M. Steegmaier, M.A. Campbell, K. Ley, H. Mossman, and D. Vestweber. 1997. The P-selectin glycoprotein ligand-1 is important for recruitment of neutrophils into inflamed mouse peritoneum. *Blood.* In press.

31. Walcheck, B., K.L. Moore, R.P. McEver, and T.K. Kishimoto. 1996. Neutrophil–neutrophil interactions under hydrodynamic shear stress involve L-selectin and PSGL-1: a mechanism that amplifies initial leukocyte accumulation on P-selectin in vitro. *J. Clin. Invest.* 98:1081–1087.

32. Tu, L.L., A.J. Chen, M.D. Delahunty, K.L. Moore, S.R. Watson, R.P. McEver, and T.F. Tedder. 1996. L-selectin binds to P-selectin glycoprotein ligand-1 on leukocytes. Interactions between the lectin, epidermal growth factor, and consensus repeat domains of the selectins determine ligand binding specificity. *J. Immunol.* 157:3995–4004.

33. Spertini, O., A.-S. Cordey, N. Monai, L. Giuffre, and M. Schapira. 1996. P-selectin glycoprotein ligand-1 (PSGL-1) is a ligand for L-selectin on neutrophils, monocytes and $CD34^+$ hematopoietic progenitor cells. *J. Cell Biol.* 135: 523–531.

34. Asa, D., L. Raycroft, L. Ma, P.A. Aeed, P.S. Kaytes, Å.P. Elhammer, and J.-G. Geng. 1995. The P-selectin glycoprotein ligand functions as a common human leukocyte ligand for P- and E-selectins. *J. Biol. Chem.* 270:11662–11670.

35. Lenter, M., A. Levinovitz, S. Isenmann, and D. Vestweber. 1994. Monospecific and common glycoprotein ligands for E- and P-selectin on myeloid cells. *J. Cell Biol.* 125:471–481.

36. Guyer, D.A., K.L. Moore, E. Lynam, C.M.G. Schammel, S. Rogelj, R.P. McEver, and L.A. Sklar. 1996. P-selectin glycoprotein ligand-1 (PSGL-1) is a ligand for L-selectin in neutrophil aggregation. *Blood.* 88:2415–2421.

37. Goetz, D.J., D.M. Greif, H. Ding, R.T. Camphausen, S. Howes, K.M. Comess, K.R. Snapp, G.S. Kansas, and F.W. Luscinskas. 1997. Isolated P-selectin glycoprotein ligand-1 dynamic adhesion to P- and E-selectin. *J. Cell Biol.* 137:509–519.

38. Patel, K.D., K.L. Moore, M.U. Nollert, and R.P. McEver. 1995. Neutrophils use both shared and distinct mechanisms to adhere to selectins under static and flow conditions. *J. Clin. Invest.* 96:1887–1896.

39. Laszik, Z., P.J. Jansen, R.D. Cummings, T.F. Tedder, R.P. McEver, and K.L. Moore. 1996. P-selectin glycoprotein ligand-1 is broadly expressed in cells of myeloid, lymphoid, and dendritic lineage and in some nonhematopoietic cells. *Blood.* 88:3010–3021.

40. Vachino, G., X.-J. Chang, G.M. Veldman, R. Kumar, D. Sako, L.A. Fouser, M.C. Berndt, and D.A. Cumming. 1995. P-selectin glycoprotein ligand-1 is the major counter-receptor for P-selectin on stimulated T cells and is widely distributed in non-functional form on many lymphocytic cells. *J. Biol. Chem.* 270:21966–21974.

41. Moore, K.L., and L.F. Thompson. 1992. P-selectin (CD62) binds to subpopulations of human memory T lymphocytes and natural killer cells. *Biochem. Biophys. Res. Commun.* 186:173–181.

42. Diacovo, T.G., S.J. Roth, C.T. Morita, J.P. Rosat, M.B. Brenner, and T.A. Springer. 1996. Interactions of human α/β and γ/δ T lymphocyte subsets in shear flow with E-selectin and P-selectin. *J. Exp. Med.* 183:1193–1203.

43. Zannettino, A.C.W., M.C. Berndt, C. Butcher, E.C. Butcher, M.A. Vadas, and P.J. Simmons. 1995. Primitive human hematopoietic progenitors adhere to P-selectin (CD62P). *Blood.* 85:3466–3477.

44. Geng, J.-G., T.J. Raub, C.A. Baker, G.A. Sawada, L. Ma, and A.P. Elhammer. 1997. Expression of a P-selectin ligand in zona pellucida of porcine oocytes and P-selectin on acrosomal membrane of porcine sperm cells. Potential implications for their involvement in sperm–egg interactions. *J. Cell Biol.* 137:743–754.

45. Weyrich, A.S., T.M. McIntyre, R.P. McEver, S.M. Prescott, and G.A. Zimmerman. 1995. Monocyte tethering by P-selectin regulates monocyte chemotactic protein-1 and tumor necrosis factor-α secretion. *J. Clin. Invest.* 95: 2297–2303.

46. Weyrich, A.S., M.R. Elstad, R.P. McEver, T.M. McIntyre, K.L. Moore, J.H. Morrissey, S.M. Prescott, and G.A. Zimmerman. 1996. Activated platelets signal chemokine synthesis by human monocytes. *J. Clin. Invest.* 97:1525–1534.

47. Patel, K.D., M.U. Nollert, and R.P. McEver. 1995. P-selectin must extend a sufficient length from the plasma membrane to mediate rolling of neutrophils. *J. Cell Biol.* 131:1893–1902.

48. Bruehl, R.E., K.L. Moore, D.E. Lorant, N. Borregaard, G.A. Zimmerman, R.P. McEver, and D.F. Bainton. 1997. Leukocyte activation induces surface redistribution of P-selectin glycoprotein ligand-1. *J. Leukocyte Biol.* 61: 489–499.

49. Lorant, D.E., R.P. McEver, T.M. McIntyre, K.L. Moore, S.M. Prescott, and G.A. Zimmerman. 1995. Activation of polymorphonuclear leukocytes reduces their adhesion to P-selectin and causes redistribution of ligands for P-selectin on their surfaces. *J. Clin. Invest.* 96:171–182.

50. Doré, M., A.R. Burns, B.J. Hughes, M.L. Entman, and C.W. Smith. 1996. Chemoattractant-induced changes in surface expression and redistribution of a functional ligand for P-selectin on neutrophils. *Blood.* 87:2029–2037.

51. Picker, L.J., R.A. Warnock, A.R. Burns, C.M. Doerschuk, E.L. Berg, and E.C. Butcher. 1991. The neutrophil selectin LECAM-1 presents carbohydrate ligands to the vascular selectins ELAM-1 and GMP-140. *Cell.* 66:921–933.

52. Bargatze, R.F., S. Kurk, E.C. Butcher, and M.A. Jutila. 1994. Neutrophils roll on adherent neutrophils bound to cytokine-induced endothelial cells via L-selectin on the rolling cells. *J. Exp. Med.* 180:1785–1792.

53. Simon, S.I., Y.P. Rochon, E.B. Lynam, C.W. Smith, D.C. Anderson, and L.A. Sklar. 1993. β2-Integrin and L-selectin are obligatory receptors in neutrophil aggregation. *Blood.* 82:1097–1106.

54. Alon, R., R.C. Fuhlbrigge, E.B. Finger, and T.A. Springer. 1996. Interactions through L-selectin between leukocytes and adherent leukocytes nucleate rolling adhesions on selectins and VCAM-1 in shear flow. *J. Cell Biol.* 135: 849–865.

55. Fuhlbrigge, R.C., R. Alon, K.D. Puri, J.B. Lowe, and T.A. Springer. 1996. Sialylated, fucosylated ligands for L-selectin expressed on leukocytes mediate tethering and rolling adhesions in physiologic flow conditions. *J. Cell Biol.* 135:837–848.

56. Ramos, C.L., B.B. Donell, K.S. Snapp, G.S. Kansas, K. Ley, and M.B. Lawrence. 1997. Myeloid cells roll on L-selectin: evidence for mucin-like ligand activity on HL-60 cells distinct from P-selectin glycoprotein ligand-1. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 11:A102. (Abstr.)

57. Zöllner, O., M.C. Lenter, J.E. Blanks, E. Borges, M. Steegmaier, H.G. Zerwes, and D. Vestweber. 1997. L-selectin from human, but not from mouse neutrophils binds directly to E-selectin. *J. Cell Biol.* 136:707–716.

58. Snapp, K.R., A.J. Wagers, R. Craig, L.M. Stoolman, and G.S. Kansas. 1997. P-selectin glycoprotein ligand-1 is essential for adhesion to P-selectin but not E-selectin in stably transfected hematopoietic cell lines. *Blood.* 89:896–901.

59. Sriramarao, P., C.R. Norton, P. Borgstrom, R.G. DiScipio, B.A. Wolitzky, and D.H. Broide. 1996. E-selectin preferentially supports neutrophil but not eosinophil rolling under conditions of flow in vitro and in vivo. *J. Immunol.* 157:4672–4680.

60. Rainger, G.E., C. Buckley, D.L. Simmons, and G.B. Nash. 1997. Cross-talk between cell adhesion molecules regulates the migration velocity of neutrophils. *Curr. Biol.* 7:316–325.

61. Haller, H., U. Kunzendorf, K. Sacherer, C. Lindschau, G. Walz, A. Distler, and F.C. Luft. 1997. T cell adhesion to P-selectin induces tyrosine phosphorylation of pp^{125} focal adhesion kinase and other substrates. *J. Immunol.* 158: 1061–1067.

62. Lin, T.H., A. Yurochko, L. Kornberg, J. Morris, J.J. Walker, S. Haskill, and R.L. Juliano. 1994. The role of protein tyrosine phosphorylation in integrin-mediated gene induction in monocytes. *J. Cell Biol.* 126:1585–1593.

63. Laudanna, C., G. Constantin, P. Baron, E. Scarpini, G. Scarlato, G. Cabrini, C. Dechecchi, F. Rossi, M.A. Cassatella, and G. Berton. 1994. Sulfatides trigger increase of cytosolic free calcium and enhanced expression of tumor necrosis factor-α and interleukin-8 mRNA in human neutrophils. Evidence for a role of L-selectin as a signaling molecule. *J. Biol. Chem.* 269:4021–4026.

64. Waddell, T.K., L. Fialkow, C.K. Chan, T.K. Kishimoto, and G.P. Downey. 1994. Potentiation of the oxidative burst of human neutrophils. A signaling role for L-selectin. *J. Biol. Chem.* 269:18485–18491.

65. Hwang, S.T., M.S. Singer, P.A. Giblin, T.A. Yednock, K.B. Bacon, S.I. Simon, and S.D. Rosen. 1996. GlyCAM-1, a physiologic ligand for L-selectin, activates β2 integrins on naive peripheral lymphocytes. *J. Exp. Med.* 184:1343–1348.

66. Brenner, B., E. Gulbins, K. Schlottman, U. Koppenhoefer, G.L. Busch, B. Walzog, M. Steinhausen, K.M. Coggeshall, O. Linderkamp, and F. Lang. 1996. L-selectin activates the Ras pathway via the tyrosine kinase $p56^{lck}$. *Proc. Natl. Acad. Sci. USA.* 93:15376–15381.

67. Waddell, T.K., L. Fialkow, C.K. Chan, T.K. Kishimoto, and G.P. Downey. 1995. Signaling functions of L-selectin. Enhancement of tyrosine phosphorylation and activation of MAP kinase. *J. Biol. Chem.* 270:15403–15411.

68. Simon, S.I., A.R. Burns, A.D. Taylor, P.K. Gopalan, E.B. Lynam, L.A. Sklar, and C.W. Smith. 1995. L-selectin (CD62L) cross-linking signals neutrophil adhesive functions via the Mac-1 (CD11b/CD18) β_2-integrin. *J. Immunol.* 155:1502–1514.

69. Norman, K.E., K.L. Moore, R.P. McEver, and K. Ley. 1995. Leukocyte rolling *in vivo* is mediated by P-selectin glycoprotein ligand-1. *Blood.* 86:4417–4421.

70. Takada, M., K.C. Nadeau, G.D. Shaw, K.A. Marquette, and N.L. Tilney. 1997. The cytokine-adhesion molecule cascade in ischemia/reperfusion injury of the rat kidney. Inhibition by a soluble P-selectin ligand. *J. Clin. Invest.* 99:2682–2690.

"Cell Adhesion In Vascular Biology"

Series Editors, Mark H. Ginsberg, Zaverio M. Ruggeri, and Ajit P. Varki

October 15, 1996	Adhesion and signaling in vascular cell–cell interactions	Guy Zimmerman, Tom McIntyre, and Stephen Prescott
November 1, 1996	Endothelial adherens junctions: implications in the control of vascular permeability and angiogenesis	Elisabetta Dejana
November 15, 1996	Genetic manipulation of vascular adhesion molecules in mice	Richard O. Hynes and Denisa D. Wagner
December 1, 1996	The extracellular matrix as a cell cycle control element in atherosclerosis and restenosis	Richard K. Assoian and Eugene E. Marcantonio
December 15, 1996	Effects of fluid dynamic forces on vascular cell adhesion	Konstantinos Konstantopoulos and Larry V. McIntire
January 1, 1997	The biology of PECAM-1	Peter J. Newman
January 15, 1997	Selectin ligands: Will the real ones please stand up?	Ajit Varki
February 1, 1997	Cell adhesion and angiogenesis	Joyce Bischoff
February 15, 1997	von Willebrand Factor	Zaverio Ruggeri
March 1, 1997	Therapeutic inhibition of carbohydrate–protein interactions in vivo	John B. Lowe and Peter A. Ward
March 15, 1997	Integrins and vascular matrix assembly	Erkki Ruoslahti
April 1, 1997	Platelet GPIIb/IIIa antagonists: The first anti-integrin receptor therapeutics	Barry S. Coller
April 15, 1997	Biomechanical activation: An emerging paradigm in endothelial adhesion biology	Michael A. Gimbrone, Jr., Tobi Nagel, and James N. Topper
May 1, 1997	Heparan sulfate proteoglycans of the cardiovascular system. Specific structures emerge but how is synthesis regulated?	Robert D. Rosenberg, Nicholas W. Shworak, Jian Liu, John J. Schwartz, and Lijuan Zhang
May 15, 1997	New insights into integrin–ligand interaction	Robert Liddington and Joseph Loftus
June 1, 1997	Adhesive interactions of sickle erythrocytes with endothelium	Robert P. Hebbel
June 15, 1997	Smooth muscle migration in atherosclerosis and restenosis	Stephen M. Schwartz
July 1, 1997	Integrin signaling in vascular biology	Sanford J. Shattil and Mark H. Ginsberg
August 1, 1997	Role of PSGL-1 binding to selectins in leukocyte recruitment	Rodger P. McEver and Richard D. Cummings