Review Article

The Biology of P-Selectin Glycoprotein Ligand-1: Its Role as a Selectin Counterreceptor in Leukocyte-Endothelial and Leukocyte-Platelet Interaction

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Summary

Cell-cell interactions mediating leukocyte trafficking, thrombogenesis and inflammation are crucial for the host defense mechanism. The selectin family of integral membrane proteins includes E-selectin, L-selectin and P-selectin. Selectins mediate tethering and rolling of leukocytes to the vessel wall at the site of inflammation. The counterreceptor for P-selectin and possibly the other selectins is P-selectin glycoprotein ligand-1 (PSGL-1). This review focuses on the properties and biology of PSGL-1.

Introduction

Cell-cell interactions mediating leukocyte trafficking, thrombogenesis and inflammation are crucial for the host defense mechanism. The selectin family of integral membrane proteins includes E-selectin (endothelial selectin), L-selectin (leukocyte selectin) and P-selectin (platelet and endothelial selectin). Selectins mediate tethering and rolling of leukocytes to the vessel wall at the site of inflammation (1). Upon contact with endothelial cells, leukocytes become activated and engaged in integrin-dependent firm attachment which leads to transendothelial migration. P-selectin expressed on activated platelets also mediates recruitment of white cells to platelets in thrombi (2). The three selectins share common structural motifs including a Ca^{+2}-dependent lectin domain, an EGF domain and a consensus repeat region. The lectin and EGF domains of selectins interact with specific glycoprotein ligands on the target cells. Oligosaccharide sequences of the ligands, predominantly sialyl Lewis X (sLex) antigen-related sialylated fucosylated lactosamine, are recognized by these selectins with considerable selectivity but low affinity (3, 4). The most challenging problem in understanding the biology of selectin ligands is to prove high affinity specific interaction with selectins under physiological conditions.

To date, a number of mucin-like proteins have been reported to bind to L-selectin and a large number of molecules, including a glycoprotein called ESL-1, have been reported to bind to E-selectin (5). These ligands have not yet been shown to mediate physiologically relevant interactions with L-selectin or E-selectin. This perspective focuses on the biology of a sialomucin molecule called P-Selectin Glycoprotein Ligand-1 (PSGL-1). PSGL-1 is the only glycoprotein thus far that seems to have a clearly defined role as a physiological ligand for P-selectin. A broader spectrum of PSGL-1 function as an L-selectin ligand and an E-selectin ligand is also suggested.

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PSGL-1 Is a Major P-Selectin Ligand in Myeloid Cells

The P-selectin ligand was originally identified in human myeloid cells as a major glycoprotein that binds to P-selectin (6-8). Subsequently identified as PSGL-1 (9), it mediates myeloid cell attachment to P-selectin under static condition or physiological shear stress (9-12). Likewise, microspheres coated with a PSGL-1-IgG chimera roll on activated endothelial cell monolayer or P-selectin expressing CHO cells (13). PSGL-1 is a minor glycoprotein on myeloid cells, presenting less than 1% of the surface sLex (10, 14). However, selective cleavage of PSGL-1 and a few other glycoproteins on myeloid cells with O-sialoglycoprotein endopeptidase without affecting overall surface expression of sLex disrupts P-selectin-specific binding (15). PSGL-1 has also been shown to play an important role in leukocyte recruitment at the site of inflammation under certain in vivo situations. Leukocyte rolling induced by inflammatory stimuli, as visualized by introvital microscopy of mouse cremaster muscle or of rat mesentery venules injected with fluorescent-labeled human polymorphonuclear neutrophils and HL-60 cells, is blocked by antibodies specific for PSGL-1 (12, 16). PSGL-1 antibody also blocks neutrophil infiltration into the inflamed peritoneum of the mouse (12).

Under hydrodynamic flow condition in vivo, PSGL-1 must bind to P-selectin with sufficient mechanical strength to sustain the interaction, yet allow rapid dissociation to facilitate rolling. Thus, the binding affinity of PSGL-1 to P-selectin must be relatively high. A Kd value of 2.4 nM and a stoichiometry of 1:1 is reported in a model system when the interaction was measured using soluble PSGL-1 and soluble P-selectin (17). As a minor glycoprotein in myeloid cells, PSGL-1 may achieve its high affinity binding to P-selectin through molecular clustering on the cell surface. The local distribution of PSGL-1 is highly enriched on the tip of the microvilli on neutrophils, eosinophils, basophils, monocytes and lymphocytes (10, 18).

PSGL-1 does not bind to P-selectin unless it is properly post-translationally modified by protein glycosylation and tyrosine sulfation (19). Binding of PSGL-1 to P-selectin requires α1,3 fucosylation, α2,3 sialylation and core-2 branched O-glycosylation (7-9, 15, 20-22). Enzymatic removal of sialic acids or O-linked glycans eliminates PSGL-1 binding to P-selectin. PSGL-1 binding to P-selectin in heterologous cells occurs only when PSGL-1 is co-expressed with certain glycosyltransferases such as α1,3 fucosyltransferase (Fuc-TIV or Fuc-TVII) in

COS cells (9), Fuc-TVII in K562 (23), or Fuc-TVII plus core-2 N-acetylglucosaminyltransferase (core-2 GnT) in CHO cells (21, 22). However, the glycan structures of PSGL-1 purified from human HL-60 cells are mostly unfucosylated. The fucosylated population of PSGL-1 includes two species of core-2 branched, sialylated and fucosylated lactosamines. The major species contains a trifucosylated polylactosamine chain and the minor species contains a short lactosamine (24). In addition to oligosaccharides, PSGL-1 contains sulfated tyrosine residues that are important for P-selectin binding (25-27). Binding of myeloid cells to P-selectin is disrupted by enzymatic removal of the sulfate moiety or blockade of sulfate synthesis. Thus, myeloid cells must express certain post-translational modification machinery in order to present PSGL-1 in a functional form.

It is noteworthy that other PSGL-1-independent mechanisms for P-selectin interaction may exist. An unknown molecule in a human colon carcinoma cell line and the heat-stable antigen CD24, a mucin-like glycosylphosphatidylinositol-linked cell surface protein expressed in many human carcinomas, have been reported to bind to P-selectin (28). These interactions may play a role in tumor metastasis by modulating tumor cell binding to platelets or endothelial cells.

Structure-Function Studies of PSGL-1

PSGL-1 was cloned and identified by expression cloning in COS cells that were co-transfected with a human HL-60 cDNA library and an α1,3/1,4 fucosyltransferase (9). The mouse homolog of PSGL-1 was subsequently cloned based on sequence similarity (11). The coding sequences of human and mouse PSGL-1 are encoded by a single exon (11, 29). The predicted protein sequence reveals a type I integral membrane protein of 402 residues for human PSGL-1 and 397 residues for mouse PSGL-1. The mature PSGL-1 protein begins at residue 42 following proteolysis of a signal peptide and cleavage of a propeptide by a furin-like converting enzyme (30). PSGL-1 purified from cell membranes is present in a homodimeric form of about 210,000 molecular weight. A disulfide bond is formed by a conserved cysteine residue near the transmembrane domain. Substitution of this cysteine residue of PSGL-1 to alanine greatly reduces the binding of PSGL-1 to P-selectin (31). Human PSGL-1 and mouse PSGL-1, although not highly conserved at the primary sequence level, share similar domain structures (Fig. 1). The most conserved region of PSGL-1 resides in the transmembrane domain and the cytoplasmic tail, consistent with a potential role in signal transduction.

Human	18 23	86	140	42	24	69	402 aa
Mouse	18 23	91	100	74	24	67	397 aa
Similarities	66 69	46	68	46	91	86	%
Identities	41 58	31	48	31	83	76	%

Sequences in the N-terminal of mature PSGL-1

Human	Q-A-T-E-**Y**$_{46}$-E-**Y**$_{48}$-L-D-**Y**$_{51}$-D-F-L- P - E - **T**$_{57}$-E - P- P-E
Mouse	Q-V-V-G-D - D-D - F-E- D -P-D-**Y**$_{54}$-**T**$_{55}$-**Y**$_{56}$-N - **T**$_{58}$-D - P-P

Consensus sequences in the repeat units of PSGL-1

Human:	Q-T-T- P -P-A-A-T-E-A
Mouse:	E-T-S-Q-P-A-P-T-E-A

Potential N-glycosylation sites in PSGL-1

Human	65, 111, 292
Mouse	66, 261

Fig. 1 Domain structure of PSGL-1 and amino acid homologies between human PSGL-1 and mouse PSGL-1. The signal peptide (S), the propeptide (P), the repeat region and the transmembrane (TM) domain are shown in the diagram. The corresponding size of each domain and the homology within each domain are shown underneath the diagram. The cysteine residue required for dimerization is indicated as * in the diagram. The sequences in the N-terminal of mature PSGL-1 and the consensus in the decameric repeat unit are given. The tyrosine residues and threonine residues that may be modified by sulfation and O-glycosylation respectively are highlighted in bold. The potential N-glycosylation sites in PSGL-1 are listed

The P-selectin binding domain of PSGL-1 is located within the first 20 residues of the mature PSGL-1 (25, 26). A recombinant Ig chimera containing residues 42-60 of human PSGL-1 is sufficient to bind to P-selectin, although not as well as the full length molecule (26). P-selectin-specific binding of human myeloid cells is inhibited by a monoclonal antibody PL1 which recognizes an epitope between residues 49-62 of human PSGL-1 (10, 32). Under physiologically relevant flow condition, PL1 also inhibited neutrophil attachment on P-selectin-coated surfaces or P-selectin expressed on CHO cells (33). Monoclonal and polyclonal antibodies against the N-terminal peptide spanning residues 42-60 of mouse PSGL-1 also block mouse myeloid cell interaction with P-selectin (11, 34).

The N-terminal binding domain of human PSGL-1 is sulfated and O-glycosylated (25-27). This binding domain of mouse and human PSGL-1 features an anionic pattern with significant difference in primary sequence. The number of tyrosine and threonine residues and their proximity to each other vary. Sulfate modification of at least one of the three tyrosine residues of human PSGL-1 is required for tight binding to P-selectin (35). A similar conclusion holds true for mouse PSGL-1. Treatment of mouse myeloid cells with arylsulfatase or sodium chlorate, an inhibitor for sulfation, abrogates adhesion of myeloid cells to P-selectin expressing CHO cells (Yang J, Galipeau J, Furie BC and Furie B, unpublished observation). Furthermore, a monoclonal antibody named KPL1 that recognizes the sulfation motif of PSGL-1 inhibited cell adhesion mediated by PSGL-1 and P-selectin (35). In addition to sulfated tyrosine residues, an O-glycan attached to threonine 57 of human PSGL-1 is also required for P-selectin binding. Although the glycosylation status of the two threonine residues in mouse PSGL-1 has not been characterized, it is likely that similar post-translational modifications occur in the mouse homolog.

PSGL-1 contains a serine/threonine- and proline-rich decameric repeat region with variable length. Mouse PSGL-1 contains ten repeat units (11). Human PSGL-1 from eosinophils (36), HL-60 and U937 cells contains 15 repeat units but PSGL-1 from human polymorphonuclear leukocytes and monocytes contains 16 repeat units (29). Like other mucin-like proteins, PSGL-1 is clustered with O-linked glycans which form highly extended structures that participate with the N-terminal binding domain of PSGL-1 to bind the counterreceptor (32). Carbohydrate modifications of PSGL-1 from human HL-60 cells are quite different from those of a structurally related mucin-like molecule CD43 (24, 37). The polypeptide backbone of PSGL-1 not only acts as a scaffold for attachment of glycans at high density but also acts to direct the synthesis and presentation of carbohydrate ligand. The repeat region of PSGL-1, however, may not directly interact with P-selectin since a monoclonal antibody PL2, which recognizes an epitope in this region, does not block PSGL-1 and P-selectin interaction (10, 32). There are two N-linked glycosylation sites predicted from the mouse PSGL-1 sequence, and three such sites are present in human PSGL-1 and at least two of them are glycosylated. N-linked glycosylation seems to have no effect on PSGL-1 binding to P-selectin since normal binding was observed even when PSGL-1 was treated with N-glycanase or when the glycosylation sites of PSGL-1 were mutagenized (8, 20, 26).

Functional PSGL-1 Is Broadly Expressed in Hematopoietic Cells

Although PSGL-1 was initially identified in myeloid cells, it is expressed in many other cell types and a subset of these cells both express PSGL-1 and interact with P- selectin (Table 1). A broad tissue distribution of PSGL-1 mRNA is observed by Northern blot analysis (9, 11). PSGL-1 mRNA is present in all hematopoietic tissues of the normal mouse, including bone marrow, spleen, thymus and lymph nodes. The highest level of PSGL-1 mRNA is found in bone marrow. However, PSGL-1 mRNA is also distributed in tissues of non-hematopoietic origin including stomach, ovary, skeletal muscle, liver, kidney, brain and adipose. Flow cytometric, immunocytochemical and immunohistochemical analyses of multiple human and mouse tissues indicated that PSGL-1 is expressed primarily in hematopoietic cells.

PSGL-1 is expressed in a functional form on circulating myeloid cells including neutrophils, monocytes, basophils and eosinophils and in circulating dendritic cells and monocyte-derived dendritic cells (10, 36, 38). Through PSGL-1, these cells interact with platelets and endothelial cells, both of which express P-selectin following their activation. PSGL-1 could be involved in recruitment of these cells to sites of inflammation, antigen exposure and allergic reaction. PSGL-1 is also widely distributed in myeloid progenitors at many stages of maturation. It remains to be shown whether PSGL-1 has a role in myeloid differentiation. In contrast, erythroid cells, megakaryocytes and platelets do not express PSGL-1 at detectable levels.

The expression pattern of PSGL-1 in lymphocytes is complex. In human and mouse lymphoid tissues including the thymus, the white pulp of the spleen, and Peyer's patches in the intestinal, PSGL-1 is expressed in T-cell enriched area (38, 39). Areas of these tissues in which B cells predominate were negative for PSGL-1. In contrast, all T and B cells in peripheral blood lymphocytes express PSGL-1 but the level of PSGL-1 in B cells is notably lower (30, 31, 35, 38). Interestingly, T cells from tonsils and T-cell zones of secondary lymphoid tissue express much less PSGL-1 than from peripheral blood, suggesting that PSGL-1 is down-regulated in T cells following extravasation into secondary lymphoid tissue. The PSGL-1 expressed in normal lymphoid tissue by T lymphocytes is, for the most part, non-functional as evaluated using a P-selectin/IgG chimera coated on fluorescent beads (39). Although comparable levels of PSGL-1 are present on naive and memory T cells, only memory T cells and particularly γ/δ cells bind to P-selectin (10, 30, 40-43). Functional PSGL-1, with all of the necessary posttranslational modifications, can also be upregulated on T cells in vitro. Antigen stimulation of T cells induces P-selectin binding, an event that correlates with the expression of core-2 GnT and fucosyltransferase activity in activated T cells (30). We speculate that PSGL-1 plays a role in primed T cell homing at sites of chronic inflammation where P-selectin is expressed. PSGL-1 expressed in naive T cells is considered non-functional solely based upon its inability to bind to P-selectin. The possibility that a second, unrelated receptor can be recognized by PSGL-1 in a distinct post-translational form cannot be eliminated.

CD34+ stem cells from human blood express PSGL-1 (38, 44, 45). These hematopoietic progenitor cells including colony-forming unit-granulocyte-macrophage (CFU-GM) and burst-forming unit-erythroid (BFU-E) bind to P-selectin and to thrombin-activated platelets (44, 45). This interaction may modulate stem cell homing, engraftment or hematopoietic cell differentiation. PSGL-1 is also expressed in immature mouse bone marrow-derived mast cells. Antibodies against the N-terminal binding domain of mouse PSGL-1 completely blocked adhesion of immature mouse bone marrow-derived mast cells to P-selectin-IgG under static or flow condition at physiological shear stress (34). PSGL-1 could mediate steady-state entry of immature mast cell into tissue and accumulation of mast cells at sites of inflammation.

Table 1 PSGL-1 distribution and correlation with P-selectin binding

Adult and Fetal Tissues	PSGL-1 Expression	P-selectin Binding
Spleen: white pulp	+(T cells)	-
red pulp	+(myeloid and macrophages)	+
Thymus: medula	+(IRDC and macrophages)	+/-
cortex	+(mostly macrophages)	+
Lymph nodes and tonsil		
T cell zone	+/-	
mantle zone and germinal center	+(FDC)	
high endothlial venule	-	-
Stomach, small and large bowels	+(macrophages in the mucosa)	+
Skin	+(epidermal Langerhans cells)	+
Liver	+(Kupfer cells)	
Lung: capilaries	+(PMN)	
alveolar	+(macrophages)	
Peripheral Blood		
Neutrophils	+	+
Monocytes	+	+
T cells	+	+(memory cells)
B cells	+	-
Basophils	+	+
Eosinophils	+	+
Platelets	-	
Erythrocytes	-	
Dendritic cells	+	+
$CD34^+$	+	+
CFU-GM, BFU-E	+	+
Mast cells (BMMC)	+	+
Bone Marrow		
Myeloblasts	+	
Erythroblasts	-	
Megakaryocytes	-	
Others		
Small colon cancer	+	+
Epithelial/fallopian tube	+	
Vascular endothelium	-	
Endothelial/chronic inflammation	+	
Zona pellucida/porcine oocytes	+	+

IRDC: interdigitating reticulum dendritic cells. FDC: follicular dendritic cells.
PMN: polymorphonuclear cells. BBMC: bone marrow derived mast cells

PSGL-1 is also found in certain non-hematopoietic cells such as epithelial cells in the fallopian tube and in some endothelial cells at sites of chronic inflammation (38). It is not clear whether these cells bind to P-selectin or any other unknown receptor. PSGL-1 has been reported on the zona pellucida of porcine oocytes, while P-selectin is present on the acrosomal membrane of porcine sperm (46). PSGL-1 mediates binding of oocytes to acrosome-reacted sperm cells in vitro. However, since the PSGL-1 nul mouse is fertile (47), there is no evidence that PSGL-1 plays any role in porcine sperm-egg interaction in vivo.

P-selectin Binding Is Reduced by Surface Redistribution of PSGL-1 following Neutrophil Activation

To facilitate dynamic rolling interaction, PSGL-1 is dispersed at the tip of microvilli on resting leukocytes including neutrophils (18). Upon activation, neutrophils disengage P-selectin-mediated rolling and utilize integrin-mediated adhesion mechanism (48). P-selectin-mediated rolling is believed to be reduced by surface redistribution of PSGL-1 to the uropods of activated neutrophils (18, 49), and is further disrupted by downregulation of P-selectin on endothelial cells. Neutrophils activated by various agonists, including PAF, fMLP, IL-8, LTB4, TNF, PMA, or calcium ionophore A23187, detach from the cell surface expressing P-selectin (18, 49). Disruption of cytoskeleton rearrangement by treatment of neutrophils with cytochalasin D before

activation prevents redistribution of PSGL-1 and reduction in P-selectin binding (49). Activated neutrophils also fail to bind to platelets (50). Similar observation of PSGL-1 redistribution and correlation with decreased P-selectin binding after activation is also reported in neutrophil-platelet interaction (51). Though evidence suggests that the level of PSGL-1 antigen is not significantly altered after neutrophil activation, changes in posttranslational modification of PSGL-1 in response to neutrophil activation might occur. The signals required to direct altered glycosylation after neutrophil activation may also be dependent on an intact cytoskeletal structure. There is, however, no universal rule correlating cell activation and reduction in P-selectin binding. Comparable P-selectin binding is found in activated monocytes (50) or phorbol ester activated CD34+ cells (45).

PSGL-1 Is an L-selectin Ligand in Neutrophils

PSGL-1 expressed on neutrophils binds to neutrophil L-selectin, mediating neutrophil rolling on other neutrophils that are already bound to activated endothelium and the initial stage of neutrophil aggregation (52-56). Antibodies specific for PSGL-1 and L-selectin block fMLP-stimulated neutrophil aggregation and interactions between myeloid cells and L-selectin-Ig chimera or L-selectin expressed on CHO cells. Purified neutrophil PSGL-1 can initiate rolling of neutrophils (57). A broader spectrum of L-selectin binding to PSGL-1 in monocytes, CD34+ hematopoietic progenitors, HL-60 and KG-1 cells has also been reported using an immunofluorescence assay with L-selectin-IgM as probe (55).

PSGL-1 adhesion to L-selectin requires the same N-terminal binding domain for P-selectin and similar post-translational modification (35, 55, 56, 58). This interaction is blocked, albeit only partially in some cases, by removal of N-terminal PSGL-1 from myeloid cells by a cobra venom metalloproteinase, treatment of myeloid cells with antibodies to the N-terminal epitope of PSGL-1, inhibition of cell sulfation, or enzymatical removal of sialylated, fucosylated O-linked glycans. However, PSGL-1 binding to L-selectin is poor under static condition. Higher levels of binding, still not comparable to P-selectin binding, are achieved under non-static condition (56). However, it is unlikely that PSGL-1 in the sole receptor involved in leukocyte-leukocyte adhesion.

PSGL-1 Binds to E-Selectin

Recently, data have suggested that regulated posttranslational modification of PSGL-1 may have a major impact on T-cell homing into inflamed skin tissues through the interaction of PSGL-1 with E-selectin (42, 59). Activated mouse CD8+ cells or human peripheral blood T cells express two forms of PSGL-1, each distinguished by the presence or absence of the cutaneous lymphocyte antigen (CLA). CLA is a carbohydrate epitope recognized by the monoclonal antibody, HECA452. T cells expressing CLA bind to both P-selectin and E-selectin, while CLA-negative cells only bind to P-selectin. HECA452-positive human peripheral blood T cells tether and roll on E-selectin and P-selectin under physiological shear stress in vitro. PSGL-1 seems to be the major glycoprotein carrier for HECA452 antigen in activated T cells. T-cell activation induces HECA452 antigen expression on PSGL-1 that correlates with the ability of purified PSGL-1 to bind to E-selectin. However, there is no direct evidence thus far to determine if HECA452-positive PSGL-1 is truly involved in T cell adhesion to E-selectin.

The binding affinity of PSGL-1 to E-selectin is at least 50-fold lower than to P-selectin as demonstrated by competitive binding studies (20). It has been well documented that E-selectin binds to sLex glycans, with a Kd between 0.1 mM and 1 mM, regardless of the polypeptide backbone. It is, therefore, not surprising to observe interactions between PSGL-1 and E-selectin in in vitro assays when PSGL-1 or E-selectin are present in high concentration. In fact, overlapping carbohydrate structures are recognized by E-selectin and P-selectin (60, 61). Core-2 branched, sialylated and fucosylated O-glycans are required for E-selectin binding (22). An IgM monoclonal antibody PL5, raised against PSGL-1 purified from HL-60, can inhibit adhesion of HL-60 cells and human neutrophils to P-selectin- and E-selectin-Ig fusion proteins (62). This IgM antibody may recognize common carbohydrate structures required for both P-selectin and E-selectin binding. However, P-selectin and E-selectin do not have identical binding sites on PSGL-1. Polyclonal antibodies raised against the N-terminal peptide, which inhibit mouse myeloid cell adhesion to P-selectin, has no effect on adhesion to E-selectin (11). The PL1 monoclonal antibody, which completely eliminates P-selectin binding to PSGL-1, only partially blocks E-selectin/PSGL-1 interaction (20). This E-selectin/PSGL-1 interaction likely involves the O-glycan attached to threonine 57 of human PSGL-1 and additional E-selectin binding site(s) on PSGL-1 may exist (13, 22, 25, 26, 33). Tyrosine sulfation has no effect on E-selectin binding. It remains unclear whether PSGL-1 binds to E-selectin on myeloid cells under physiological conditions.

PSGL-1 Signals Biological Responses in Leukocytes

PSGL-1 on leukocyte surface is not only an adhesion molecule that supports cell-cell recognition, it may also transmit signals directly into leukocytes. Cross-linking of PSGL-1 on mouse neutrophils by P-selectin induces Mac-1 expression and enhanced beta 2-integrin-dependent neutrophil adhesion to ICAM-1 (63). Human neutrophils, however, do not exhibit the same properties. PSGL-1 blockade abrogates P-selectin-mediated biochemical responses in activated monocytes and platelets. Binding of P-selectin to monocytes is sufficient to induce tissue factor activity (64). Tissue factor expression can be greatly accelerated by a combination of P-selectin and other small molecules such as 12-HETE [Pelligrini, submitted]. Different cytokines are induced when monocytes bind P-selectin through PSGL-1 and are primed by several different synergistic activators. Exposure of monocytes to P-selectin and PAF mobilizes the transcription factor NF-kB and induces expression of the cytokines TNFa and MCP-1, monocyte chemotactic protein-1 (65). Monocytes exposed to P-selectin and the platelet-derived chemokines, RANTES, secrete a different set of cytokines including IL-8 and MCP-1 (66).

The signal pathways transmitted by PSGL-1 remain unclear. However, information regarding tyrosine phosphorylation in response to PSGL-1 interaction with P-selectin has been reported. Tyrosine phosphorylation of pp125 focal adhesion kinase (FAK) is induced when T cells bind to P-selectin (67). Cross-linking of neutrophil PSGL-1 with monoclonal antibodies or immobilized P-selectin induces rapid tyrosine phosphorylation of the ERK family of mitogen-activated protein kinases and secretion of IL-8. IL-8 secretion is blocked by a tyrosine kinase inhibitor (68).

PSGL-1 Plays a Pathological Function in Certain Disease Models

PSGL-1 is a physiologically important ligand for selectins. In animal models, PSGL-1 plays a role in pathological leukocyte recruitment in certain disease models. Recruitment of mouse T helper 1 cells into

inflamed sites of mouse skin in a delayed hypersensitivity model is blocked by antibodies against the N-terminal peptide of mouse PSGL-1 (43). PSGL-1 antibody also inhibits neutrophil accumulation in mouse peritoneum in a peritoneal inflammation model induced by thioglycolate (12). In an ischemia/reperfusion injury model, infusion of soluble PSGL-1 blocks acute inflammation events in rat kidney (69). Soluble PSGL-1 also inhibited adjuvant-induced arthritis in the Lewis rat (70).

PSGL-1 should also contribute to pathological effects of inflammatory and thrombotic disorders in which P-selectin is implicated. Studies from P-selectin knock-out mice indicated a role of P-selectin/PSGL-1 interaction in a model of cytokine-induced meningitis (71) and atherosclerosis in mice under high fat diet (72). In rat models, P-selectin binding to PSGL-1 is involved in neutrophil infiltration in rat lung injury induced by cobra venom factor (73) or induced by intrapulmonary deposition of IgG immune complexes (74), and in neutrophil deposition within tissues in response to intravenous administration of lipopolysaccharide (75). A possible role of P-selectin and PSGL-1 interaction in thrombosis is also suggested in a primate model in which adhesion of leukocytes at the wound generated by a Dracron graft and fibrin deposition on the graft is prevented by P-selectin antibodies (2). A role of PSGL-1 in cell malignancy and in allergic reaction may also be postulated since functional PSGL-1 that binds to P-selectin is found in tumor cells (76) and eosinophils (10, 18).

Conclusion

PSGL-1 is an adhesion molecule that is involved in multiple cellular interactions and biological responses during leukocyte recruitment. This mucin-like protein may interact with each of the three selectins under certain physiological conditions. With the recent generation of a viable PSGL-1 nul mouse (47), the examination of inflammation, thrombosis and immune function in this animal model should provide detailed information about the physiological role of PSGL-1.

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Received May 7, 1998 Accepted after revision September 29, 1998