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Tyrosine Sulfation of P-selectin Glycoprotein Ligand-1 Is Required for High Affinity Binding to P-selectin*

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Patricia P. Wilkins‡, Kevin L. Moore§¶‖, Rodger P. McEver‡§¶, and Richard D. Cummings‡ ‡‡**

*From the Departments of ‡Biochemistry and Molecular Biology and ¶Medicine, 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 73190*

P-selectin glycoprotein ligand-1 (PSGL-1) is a mucin-like glycoprotein on leukocytes that is a high affinity ligand for P-selectin. Previous studies have shown that sialylation and fucosylation of PSGL-1 are required for its binding to P-selectin, but other post-translational modifications of PSGL-1 may also be important. We demonstrate that PSGL-1 synthesized in human HL-60 cells can be metabolically labeled with [^{35}S]sulfate that is incorporated primarily into tyrosine sulfate. Treatment of PSGL-1 with a bacterial arylsulfatase releases sulfate from tyrosine, resulting in a concordant decrease in binding to P-selectin. These studies demonstrate that tyrosine sulfate on PSGL-1 functions in conjunction with sialylated and fucosylated glycans to mediate high affinity binding to P-selectin.

P-selectin is a calcium-dependent carbohydrate-binding protein that is expressed on the surfaces of activated platelets and endothelium in response to thrombin and other agonists (1–3). Through its binding to glycoconjugate-based counter-receptors on leukocytes, P-selectin mediates rolling adhesion of these cells on activated platelets and endothelium (4, 5). Both sialic acid and fucose are components of the P-selectin counter-receptors on leukocytes (6–8). Oligosaccharides containing sialyl Lewis x (sLe^x),[1] NeuAcα2–3Galβ1–4[Fucα1–3]GlcNAcβ1-R, a determinant present on leukocyte surfaces, inhibit adhesion of leukocytes to P-selectin (9, 10). However, expression of sLe^x on cell surfaces is not sufficient for high affinity binding of cells to P-selectin, since non-myeloid cells that express high levels of sLe^x bind poorly to P-selectin compared to myeloid cells (11).

Leukocytes express a single high affinity ligand for P-selectin, termed P-selectin glycoprotein ligand-1 (PSGL-1) (5, 7, 8, 12, 13). PSGL-1 is a homodimeric glycoprotein with two disulfide-bonded 120-kDa subunits (7). The cDNA-derived sequence for PSGL-1 predicts a type 1 transmembrane protein of 402 amino acids (8). The extracellular domain has an N-terminal signal peptide from residues 1–18 and a putative propeptide from residues 19–41. Assuming cleavage of the propeptide, the extracellular domain of the mature protein begins at residue 42 and extends to residue 308. The sequence concludes with a 25-residue transmembrane domain and a 69-residue cytoplasmic tail. The extracellular domain is rich in serines and threonines that are potential sites for *O*-glycosylation. There are also three potential *N*-glycosylation sites and three potential tyrosine sulfation sites at residues 46, 48, and 51 (8).

PSGL-1 must be sialylated and fucosylated to bind P-selectin (7, 8). Consistent with these observations, PSGL-1 is highly *O*-glycosylated (12) and contains sialylated and fucosylated *O*-linked poly-*N*-acetyllactosamine, including some glycans that terminate in sLe^x (13). It is not clear, however, that sLe^x or related glycans are sufficient for high affinity binding of PSGL-1 to P-selectin. For example, sulfated compounds lacking either sialic acid or fucose can inhibit adhesion of leukocytes to P-selectin (14–17). These data suggest that PSGL-1 may require sulfation to bind with high affinity to P-selectin. We demonstrate that PSGL-1 is sulfated, primarily on tyrosine residues. Furthermore, tyrosine sulfation of PSGL-1 is required for high affinity binding to P-selectin.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents—Carrier-free [^{35}S]sulfate (1100–1600 mCi/mmol) was purchased from DuPont NEN. Emphaze™ affinity support resin was purchased from Pierce. *Aerobacter aerogenes* arylsulfatase, *Arthrobacter ureafaciens* neuraminidase, and sulfated monosaccharides were obtained from Sigma. Recombinant peptide:*N*-glycosidase F was purchased from Boehringer Mannheim. All cell culture reagents were obtained from Life Technologies, Inc. Authentic tyrosine sulfate was synthesized as described (18) using concentrated H_2SO_4 and tyrosine. Other chemicals were ACS grade or better and were obtained from Fisher Scientific.

Isolation of Radiolabeled PSGL-1—PSGL-1 was purified from human neutrophils and radiolabeled with Na^{125}I as described (13). HL-60 cells (2–5 × 10^5 cells/ml) were labeled for 48 h with 100 μCi/ml [^{35}S]sulfate at 37 °C in sulfate-deficient medium containing 10% dialyzed fetal bovine serum. ^{3}H-PSGL-1 was prepared by metabolic labeling of HL-60 cells with [^{3}H]glucosamine as described (7, 12). ^{3}H-PSGL-1 and ^{35}S-PSGL-1 were purified using affinity chromatography with a column containing recombinant soluble P-selectin (19) coupled to Emphaze™ at a density of 5 mg/ml (13). The enriched EDTA-eluted samples were rechromatographed on the P-selectin column after dialysis into Ca^{2+}-containing buffer. Purified ^{35}S-PSGL-1 was treated with reducing or nonreducing SDS sample buffer and electrophoresed in a 7.5% polyacrylamide gel (20). Gels were dried and then exposed to Fuji RX x-ray film at −80 °C.

Detection of Tyrosine Sulfate—Tyrosine sulfation of PSGL-1 was determined by base hydrolysis of ^{35}S-PSGL-1. Following identification of ^{35}S-PSGL-1 by autoradiography, the dried gel was aligned with the exposed x-ray film, and the band from the nonreducing lane was subjected to strong base hydrolysis in 1.0 M NaOH at 110 °C, for 24 h as described (21). The hydrolysate was passed over a Dowex 50 (H^+) column washed with water, lyophilized, and analyzed by anion exchange chromatography using a Varian AX-5 column (4 mm × 30 cm) that was eluted with a gradient of NaH_2PO_4, pH 3.0. Tyrosine sulfate was also detected using descending paper chromatography as described

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‡‡ To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, 941 S. L. Young Blvd., Oklahoma City, OK 73190. Tel.: 405-271-2481; Fax: 405-271-3910.

[1] The abbreviations used are: sLe^x, sialyl Lewis x; PSGL-1, P-selectin glycoprotein ligand-1; PAGE, polyacrylamide gel electrophoresis; NRS, normal rabbit serum; HPLC, high pressure liquid chromatography.

(22). Briefly, authentic standards were separated on Whatman No. 1 cellulose paper using 1-butanol:acetic acid:water (125:30:125) for 18 h. Tyrosine and tyrosine sulfate were detected with 0.25% ninhydrin/acetone, and sulfated monosaccharides were detected by silver stain (23).

Enzymatic Treatment of PSGL-1—All arylsulfatase digestions were performed with *A. aerogenes* arylsulfatase (EC 3.1.6.1) (24) in approximately 500 μl of 0.01 M Tris-HCl, pH 7.5, overnight at 37 °C. Following digestion, the enzyme reactions were terminated by boiling for 15 min. Sham digestions were performed with 1000 milliunits of boiled enzyme. [^{35}S]Tyrosine sulfate from ^{35}S-PSGL-1 was prepared by base hydrolysis as described above. The base hydrolysate was treated with 50 or 1000 milliunits of active arylsulfatase or with 1000 milliunits of boiled arylsulfatase, then analyzed by anion exchange chromatography.

The specificity of the *A. aerogenes* arylsulfatase was determined for the sulfated monosaccharides GlcNAc-6-sulfate, Gal-6-sulfate, GalNAc-6-sulfate, and GalNAc-4-sulfate. Each of the sulfated monosaccharides (100 nmol) was treated separately with 1000 milliunits of boiled or active enzyme. After overnight incubation, the reaction mixtures were boiled for 15 min, lyophilized, resuspended in water, and analyzed by high pH anion exchange chromatography with a Dionex CarboPac PA-1 column and PAD detection as described (25).

Intact ^{35}S-PSGL-1, ^{125}I-PSGL-1, or ^{3}H-PSGL-1 was treated with *A. aerogenes* arylsulfatase as described above. The [^{35}S]sulfate released from ^{35}S-PSGL-1 by arylsulfatase was quantified by $BaSO_4$ precipitation, in which 100 μl of saturated $BaCl_2$ was added to the reaction mixture with 10 μl of saturated Na_2SO_3 as a carrier (26). In the graphic representation of the data, the sulfate released from the sham-treated sample was adjusted setting the sham-treated sample equal to 0%. The actual percentage of radioactivity released from the sham-treated samples ranged from 1.3–5.0%.

^{35}S-PSGL-1 was treated with peptide:*N*-glycosidase F as described (26). ^{35}S-PSGL-1 was denatured by boiling 5 min in 50 μl of 50 mM sodium phosphate, pH 7.5, 50 mM 2-mercaptoethanol, 0.5% SDS. The SDS was then diluted to a final concentration of 0.2% with 1.5% Nonidet P-40. 10 units of enzyme was added to the denatured ^{35}S-PSGL-1 and incubated overnight at 37 °C.

In a control experiment, ^{125}I-PSGL-1 was treated with 100 milliunits of *A. ureafaciens* neuraminidase in 0.05 M sodium acetate, pH 5.0, overnight at 37 °C prior to application to a CSLEX-1 antibody column. Chromatography on CSLEX-1 was performed as described previously (13).

Rebinding of Arylsulfatase-treated PSGL-1 to P-selectin—Arylsulfatase-treated ^{125}I-PSGL-1 and ^{3}H-PSGL-1 were applied to a P-selectin affinity column in Ca^{2+}-containing buffer, and the bound radioactivity was eluted with EDTA. The percentage of PSGL-1 eluted from the column with EDTA was corrected setting the sham-treated sample equal to 100% (actual rebinding ranged from 87–99%).

Immunoprecipitation and P-selectin Binding Assays—Immunoprecipitation of ^{35}S-PSGL-1 was performed as described (13) using a rabbit antiserum (5, 13) to a peptide corresponding to residues 42–56 (QATEYEYLDYDFLPE) of PSGL-1 (8) (anti-42–56) or normal rabbit serum (NRS). The immunoprecipitates were analyzed by SDS-PAGE under nonreducing conditions, followed by autoradiography.

RESULTS AND DISCUSSION

To determine if PSGL-1 is post-translationally sulfated, human promyelocytic leukemia HL-60 cells were metabolically labeled with [^{35}S]sulfate, and PSGL-1 was purified from lysates of these cells by affinity chromatography on a P-selectin column. A [^{35}S]sulfate-labeled protein was detected that migrated in SDS gels with a relative molecular mass of 120,000 under reducing conditions and 240,000 under nonreducing conditions (Fig. 1). These mobilities are consistent with the disulfide-linked homodimeric structure of PSGL-1 (7). Furthermore, the [^{35}S]sulfate-labeled protein was immunoprecipitated with a specific rabbit antiserum generated against a synthetic peptide encoding residues 42–56 of the extracellular domain of PSGL-1 (13) (Fig. 1). Analysis of the supernatants showed that anti-42–56 precipitated all of the ^{35}S-PSGL-1, whereas NRS precipitated none of the ^{35}S-PSGL-1 (data not shown). These results demonstrate that PSGL-1 is post-translationally sulfated.

Sulfate can be incorporated into eukaryotic glycoproteins as tyrosine sulfate (27) or as sulfated carbohydrates (28, 29). In preliminary studies we failed to detect sulfated carbohydrates

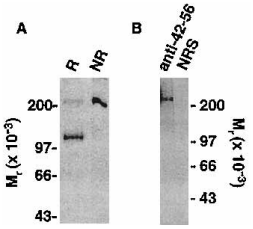

FIG. 1. **PSGL-1 is post-translationally sulfated.** *A*, affinity-purified ^{35}S-PSGL-1 from HL-60 cells was resolved by SDS-PAGE under reducing (*R*) and nonreducing (*NR*) conditions, followed by autoradiography. *B*, the purified ^{35}S-PSGL-1 was immunoprecipitated with a specific rabbit antiserum to PSGL-1 (anti-42–56) or with normal rabbit serum (NRS). The immunoprecipitates were electrophoresed under nonreducing conditions.

on PSGL-1, using techniques that detected such structures on other glycoproteins (25, 26). We then considered the possibility that PSGL-1 might contain tyrosine sulfate. The cDNA sequence of PSGL-1 predicts four extracytoplasmic tyrosine residues (8), three of which are clustered at positions 46, 48, and 51 within a predicted consensus sequence for tyrosine sulfation (27). To determine if PSGL-1 has tyrosine sulfate, the gel slice containing the 240-kDa ^{35}S-PSGL-1 was hydrolyzed with strong base and analyzed by both anion exchange chromatography and descending paper chromatography. In both systems, a single radioactive peak was recovered that comigrated with authentic tyrosine sulfate (Fig. 2, *A* and *B*). No radioactivity was recovered that comigrated with sulfated monosaccharide standards.

We sought to test whether tyrosine sulfate is important for binding of PSGL-1 to P-selectin. Although the functions of tyrosine sulfate within proteins are not clear (27), some proteins require this modification for optimal activity (30, 31). The usual approach for assessing the importance of tyrosine sulfate is to prevent sulfation of newly synthesized proteins with chemical inhibitors or to replace tyrosine with phenylalanine by site-directed mutagenesis. We developed an alternative approach in which sulfate was enzymatically removed from tyrosine on intact PSGL-1. We first tested the ability of an arylsulfatase from *A. aerogenes* to release sulfate from [^{35}S]tyrosine sulfate. Treatment of the hydrolysate of ^{35}S-PSGL-1 with 1000 milliunits of arylsulfatase quantitatively released [^{35}S]sulfate from [^{35}S]tyrosine sulfate (Fig. 3, *A* and *B*). In other experiments, as little as 50 milliunits of this arylsulfatase also quantitatively released [^{35}S]sulfate from [^{35}S]tyrosine sulfate derived from PSGL-1 (data not shown). Cleavage by arylsulfatase was specific, since 1000 milliunits of the enzyme did not release sulfate from Gal-6-sulfate, GlcNAc-6-sulfate, GalNAc-4-sulfate, and GalNAc-6-sulfate (data not shown).

We then examined the ability of the arylsulfatase to release sulfate from intact PSGL-1 and the effect of this release on rebinding of PSGL-1 to P-selectin. [^{35}S]Sulfate released from ^{35}S-PSGL-1 by arylsulfatase was quantified by precipitation as insoluble $BaSO_4$. Up to 50% of the [^{35}S]sulfate on ^{35}S-PSGL-1 was released by 500 milliunits of arylsulfatase; increasing amounts of enzyme did not release more radioactivity (Fig. 4*A*). The functional importance of the tyrosine sulfate on PSGL-1 was assessed by treating both ^{3}H-PSGL-1 from HL-60 cells and ^{125}I-PSGL-1 from human neutrophils with arylsulfatase and measuring the rebinding of the treated ligands to a P-selectin column. Binding of both arylsulfatase-treated ^{3}H-PSGL-1 and ^{125}I-PSGL-1 to P-selectin was reduced in a dose-dependent manner; the decreased binding was inversely related to the amount of sulfate released from ^{35}S-PSGL-1 (Fig. 4*A*). The reduced binding of PSGL-1 to P-selectin following arylsulfatase treatment was not due to general release of sialic acid and/or

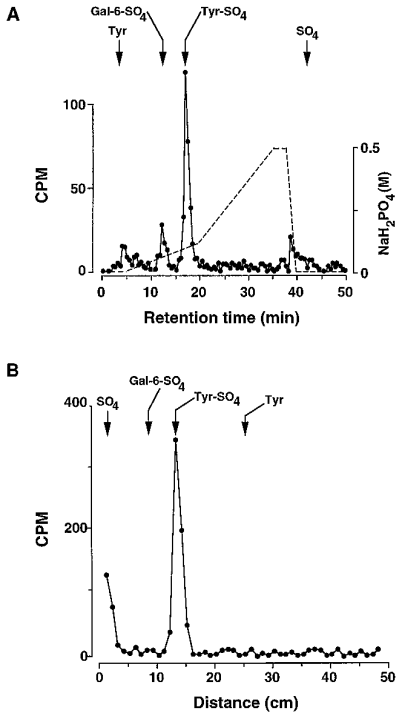

FIG. 2. **PSGL-1 contains tyrosine sulfate.** ^{35}S-PSGL-1 was hydrolyzed with strong base as described under "Experimental Procedures." *A*, the hydrolysate was analyzed by anion exchange chromatography using HPLC with a NaH_2PO_4, pH 3.0 gradient (*dashed line*). The retention times of tyrosine, tyrosine sulfate, Gal-6-sulfate, and free sulfate are indicated. Other sulfated monosaccharides (GlcNAc-6-sulfate, GalNAc-6-sulfate, and GalNAc-4-sulfate) eluted with similar retention times between 14 and 15 min. *B*, the hydrolysate was analyzed by descending paper chromatography in the solvent system 1-butanol: acetic acid:water (125:30:125) for 18 h. The migration distances for tyrosine, tyrosine sulfate, Gal-6-sulfate, and free sulfate are indicated. The R_f values for tyrosine, tyrosine sulfate, sulfated monosaccharides (Gal-6-sulfate, GlcNAc-6-sulfate, GalNAc-6-sulfate, and GalNAc-4-sulfate), and free sulfate were 0.5, 0.28, 0.12–0.15, and 0.04, respectively.

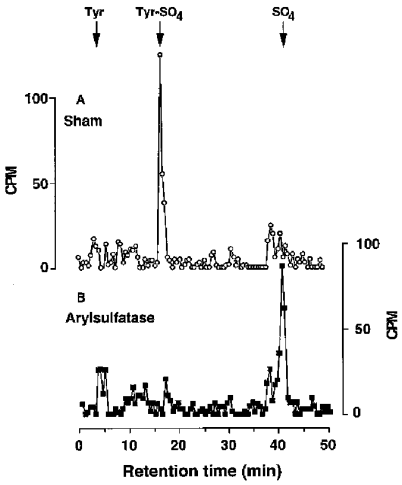

FIG. 3. **Tyrosine sulfate from PSGL-1 is sensitive to arylsulfatase.** ^{35}S-PSGL-1 was hydrolyzed with strong base as described under "Experimental Procedures." The hydrolysates were either sham-treated with 1000 milliunits of boiled arylsulfatase (*A*) or treated with 1000 milliunits of active enzyme (*B*). The hydrolysates were then analyzed by anion exchange chromatography.

fucose by contaminating exoglycosidases, because PSGL-1 treated with 1000 milliunits of arylsulfatase bound quantitatively to an affinity column containing CSLEX-1, a monoclonal antibody to sLe^x (data not shown).

Arylsulfatase released ≈50% of the [^{35}S]sulfate from ^{35}S-PSGL-1 and decreased rebinding of ^{3}H-PSGL-1 and ^{125}I-PSGL-1 to P-selectin by the same degree. We considered the possibility that the fraction of ^{35}S-PSGL-1 that rebound to P-selectin following treatment with arylsulfatase retained critical tyrosine sulfate residues, whereas the fraction that did not rebind to P-selectin had lost tyrosine sulfate. ^{35}S-PSGL-1 was treated with 1000 milliunits of arylsulfatase and then applied to a P-selectin column. The bound and unbound fractions were analyzed by SDS-PAGE (Fig. 4*B*). A band corresponding to ^{35}S-PSGL-1 was observed in the bound fractions, whereas no band was seen in the unbound fractions. The radioactivity in the unbound fractions represented free sulfate. When the band of ^{35}S-PSGL-1 in the bound fractions was hydrolyzed, radioactivity was recovered in tyrosine sulfate (data not shown). In the control experiment, ^{125}I-PSGL-1 was treated with 1000 milliunits of arylsulfatase and then applied to the P-selectin column. SDS-PAGE analysis revealed that intact ^{125}I-PSGL-1 was recovered in both the unbound and bound fractions (Fig. 4*B*). Thus, arylsulfatase removed sulfate quantitatively from a subset of PSGL-1, which no longer bound P-selectin, but the enzyme did not otherwise degrade PSGL-1.

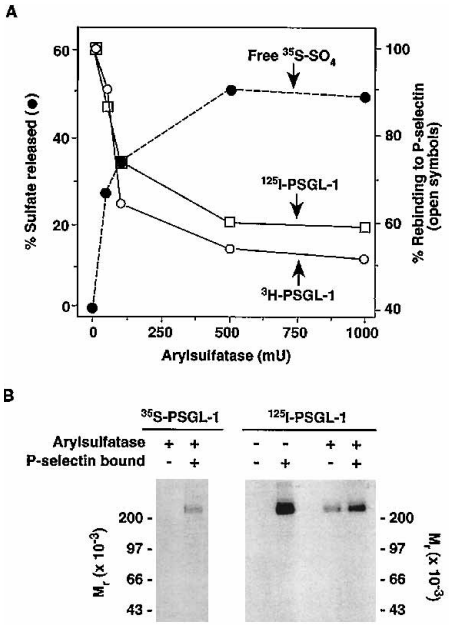

FIG. 4. **Tyrosine sulfate is important for P-selectin binding.** *A*, arylsulfatase releases [^{35}S]sulfate from intact ^{35}S-PSGL-1 and decreases Ca^{2+}-dependent binding of both ^{125}I-PSGL-1 and ^{3}H-PSGL-1 to P-selectin. ^{35}S-PSGL-1 and ^{3}H-PSGL-1 purified from HL-60 cells and ^{125}I-PSGL-1 purified from human neutrophils were treated with increasing amounts of arylsulfatase. The [^{35}S]sulfate released from ^{35}S-PSGL-1 was quantified using $BaSO_4$ precipitation (*dashed line*). Enzyme-treated ^{125}I-PSGL-1 and ^{3}H-PSGL-1 were analyzed for their ability to rebind to P-selectin (*solid lines*). *B*, [^{35}S]tyrosine sulfate is present on PSGL-1 that binds P-selectin but is absent on PSGL-1 that does not bind P-selectin. Arylsulfatase-treated ^{35}S-PSGL-1 and ^{125}I-PSGL-1 were applied to a P-selectin column. The unbound (−) and bound (+) fractions were analyzed by SDS-PAGE under nonreducing conditions, followed by autoradiography.

The tyrosine sulfate remaining in the P-selectin-bound subset of PSGL-1 may be resistant to arylsulfatase because of its inaccessibility or because of some other feature of PSGL-1 that blocks action of the enzyme. When ^{35}S-PSGL was partly deglycosylated by treatment with peptide:*N*-glycosidase F and *A. ureafaciens* neuraminidase, subsequent treatment with arylsulfatase released up to 70% of the radioactivity as [^{35}S]sulfate (data not shown). Since neuraminidase also eliminates binding of PSGL-1 to P-selectin, we could not determine whether the increased removal of [^{35}S]sulfate further reduced binding of PSGL-1 to P-selectin. These results suggest that the extensive

glycosylation of PSGL-1 may account for the inaccessibility of some tyrosine sulfate sites to arylsulfatase.

These results demonstrate that PSGL-1 contains tyrosine sulfate that is required for high affinity binding to P-selectin. It has been shown previously that PSGL-1 contains the sLe^x determinant on *O*-linked oligosaccharides and that both sialic acid and fucose are required for binding of PSGL-1 to P-selectin (7, 8, 12, 13). Tyrosine sulfate may be important because it promotes appropriate presentation of the glycans that bind directly to P-selectin. Alternatively, tyrosine sulfate may directly interact with P-selectin. This latter possibility seems more likely, since sulfatide and sulfated oligosaccharides are known to bind P-selectin (14–17). Sulfate is also a critical determinant for the binding of GlyCAM-1 to L-selectin, but GlyCAM-1 contains sulfate in Gal-6-sulfate and GlcNAc-6-sulfate (32–34). In contrast, PSGL-1 is sulfated primarily on tyrosine rather than on monosaccharides.

Our results suggest a model in which PSGL-1 presents both carbohydrate and tyrosine sulfate as components of a critical recognition site for P-selectin. We hypothesize that this site is located at the N-terminal, membrane-distal region of PSGL-1, near the three potentially sulfated tyrosine residues. Consistent with this hypothesis, PL1, a monoclonal antibody that recognizes a membrane-distal epitope on PSGL-1, blocks binding of fluid-phase P-selectin to leukocytes and abolishes adhesion of neutrophils to P-selectin under both static and shear conditions (5). In contrast, PL2, a monoclonal antibody that recognizes a membrane-proximal epitope on PSGL-1, does not inhibit binding to P-selectin (5). The concept that P-selectin recognizes a localized site on a mucin-like glycoprotein is in contrast to a model in which a selectin recognizes multiple, clustered *O*-linked glycans attached along the entire polypeptide (35, 36).

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REFERENCES

1. McEver, R. P., Moore, K. L., and Cummings, R. D. (1995) *J. Biol. Chem.* **270,** 11025–11028
2. Varki, A. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 7390–7397
3. Springer, T. A. (1995) *Annu. Rev. Physiol.* **57,** 827–872
4. Lawrence, M. B., and Springer, T. A. (1991) *Cell* **65,** 852–873
5. Moore, K. L., Patel, K. D., Bruehl, R. E., Li, F., Johnson, D. A., Lichenstein, H. S., Cummings, R. D., Bainton, D. F., and McEver, R. P. (1995) *J. Cell Biol.* **128,** 661–671
6. Corral, L., Singer, M. S., Macher, B. A., and Rosen, S. D. (1990) *Biochem. Biophys. Res. Commun.* **172,** 1349–1356
7. Moore, K. L., Stults, N. L., Diaz, S., Smith, D. F., Cummings, R. D., Varki, A., and McEver, R. P. (1992) *J. Cell Biol.* **118,** 445–456
8. Sako, D., Chang, X.-J., Barone, K. M., Vachino, G., White, H. M., Shaw, G., Veldman, G. M., Bean, K. M., Ahern, T. J., Furie, B., Cumming, D., and Larsen, G. R. (1993) *Cell* **75,** 1179–1186
9. Polley, M. J., Phillips, M. L., Wayner, E., Nudelman, E., Singhal, A. K., Hakomori, S.-I., and Paulson, J. C. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 6224–6228
10. Foxall, C., Watson, S. R., Dowbenko, D., Fennie, C., Lasky, L. A., Kiso, M., Hasegawa, A., Asa, D., and Brandley, B. K. (1992) *J. Cell Biol.* **117,** 895–902
11. Zhou, Q., Moore, K. L., Smith, D. F., Varki, A., McEver, R. P., and Cummings, R. D. (1991) *J. Cell Biol.* **115,** 557–564
12. Norgaard, K. E., Moore, K. L., Diaz, S., Stults, N. L., Ushiyama, S., McEver, R. P., Cummings, R. D., and Varki, A. (1993) *J. Biol. Chem.* **268,** 12764–12774
13. Moore, K. L., Eaton, S. F., Lyons, D. E., Lichenstein, H. S., Cummings, R. D., and McEver, R. P. (1994) *J. Biol. Chem.* **269,** 23318–23327
14. Aruffo, A., Kolanus, W., Walz, G., Fredman, P., and Seed, B. (1991) *Cell* **67,** 35–44
15. Nelson, R. M., Cecconi, O., Roberts, W. G., Aruffo, A., Linhardt, R. J., and Bevilacqua, M. P. (1993) *Blood* **82,** 3253–3258
16. Cecconi, O., Nelson, R. M., Roberts, W. G., Hanasaki, K., Mannori, G., Schultz, C., Ulich, T. R., Aruffo, A., and Bevilacqua, M. P. (1994) *J. Biol. Chem.* **269,** 15060–15066
17. Skinner, M. P., Fournier, D. J., Andrews, R. K., Gorman, J. J., Chesterman, C. N., and Bernt, M. C. (1989) *Biochem. Biophys. Res. Commun.* **164,** 1373–1379
18. Huttner, W. B. (1984) *Methods Enzymol.* **107,** 200–224
19. Ushiyama, S., Laue, T. M., Moore, K. L., Erickson, H. P., and McEver, R. P. (1993) *J. Biol. Chem.* **268,** 15229–15237
20. Laemmli, U. K. (1970) *Nature* **227,** 680–685
21. Hortin, G., Tollefsen, D. M., and Strauss, A. W. (1986) *J. Biol. Chem.* **261,** 15827–15830
22. Dodgson, K. S., Rose, F. A., and Tudball, N. (1959) *Biochem. J.* **71,** 10–15
23. Cummings, R. D., Kornfeld, S., Schneider, W. J., Hobgood, K. K., Tolleshaug, H., Brown, M. S., and Goldstein, J. L. (1983) *J. Biol. Chem.* **258,** 15261–15273
24. Fowler, L. R., and Rammler, D. H. (1964) *Biochemistry* **3,** 230–237
25. Shilatifard, A., and Cummings, R. D. (1995) *Glycobiology* **5,** 291-297
26. Shilatifard, A., Merkle, R. M., Helland, D. E., Welles, J. L., Haseltine, W. A., and Cummings, R. D. (1993) *J. Virol.* **67,** 943–952
27. Huttner, W. B., and Baeuerle, P. A. (1988) *Mod. Cell Biol.* **6,** 97–140
28. Fiete, D., Srivastava, V., Hindsgaul, O., and Baenziger, J. (1991) *Cell* **65,** 1103–1110
29. Kjellan, L., and Lindahl, U. (1991) *Annu. Rev. Biochem.* **60,** 443–475
30. Pittman, D. D., Wang, J. H., and Kaufman, R. J. (1992) *Biochemistry* **31,** 3315–3325
31. Dong, J.-F., Li, C. Q., and Lopez, J. A. (1994) *Biochemistry* **33,** 13946–13953
32. Imai, Y., Lasky, L. A., and Rosen, S. D. (1993) *Nature* **361,** 555–557
33. Hemmerich, S., Bertozzi, C. R., Leffler, H., and Rosen, S. D. (1994) *Biochemistry* **33,** 4820–4829
34. Hemmerich, S., Leffler, H., and Rosen, S. D. (1995) *J. Biol. Chem.* **270,** 12035–12047
35. Lasky, L. A., Singer, M. S., Dowbenko, D., Imai, Y., Henzel, W. J., Grimley, C., Fennie, C., Gillett, N., Watson, S. R., and Rosen, S. D. (1992) *Cell* **69,** 927–938
36. Baumheuter, S., Singer, M. S., Henzel, W., Hemmerich, S., Renz, M., Rosen, S. D., and Lasky, L. A. (1993) *Science* **262,** 436–438