A Novel Cobra Venom Metalloproteinase, Mocarhagin, Cleave a 10-Amino Acid Peptide from the Mature N Terminus of P-selectin Glycoprotein Ligand Receptor, PSGL-1, and Abolishes P-selectin Binding*

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Initial rolling of circulating neutrophils on a blood vessel wall prior to adhesion and transmigration to damaged tissue is dependent upon P-selectin expressed on endothelial cells and its specific neutrophil receptor, the P-selectin glycoprotein ligand-1 (PSGL-1). Pretreatment of neutrophils, HL60 cells, or a recombinant fusocyslated soluble form of PSGL-1 (sPSGL-1.T7) with the cobra venom metalloproteinase, mocarhagin, completely abolished binding to purified P-selectin in a time-dependent and EDTA- and diisopropyl fluorophosphate-inhibitable manner consistent with mocarhagin selectively cleaving PSGL-1. A polyclonal antibody against the N-terminal peptide Gln-1-Glu-15 of mature PSGL-1 immunoprecipitated sPSGL-1.T7 but not sPSGL-1.T7 treated with mocarhagin, indicating that the mocarhagin cleavage site was near the N terminus. A single mocarhagin cleavage site between Tyr-10 and Asp-11 of mature PSGL-1 was determined by N-terminal sequencing of mocarhagin fragments of sPSGL-1.T7 and is within a highly negatively charged amino acid sequence QATEYELDY DFLPETEPPE, containing three tyrosine residues that are consensus sulfation sites. Consistent with a functional role of this region of PSGL-1 in binding P-selectin, an affinity-purified polyclonal antibody against residues Gln-1-Glu-15 of PSGL-1 strongly inhibited P-selectin binding to neutrophils, whereas an antibody against residues Asp-9-Arg-23 was noninhibitory. These combined data strongly suggest that the N-terminal anionic/sulfated tyrosine motif of PSGL-1 as well as downstream sialylated carbohydrate is essential for binding of P-selectin by neutrophils.

In response to inflammatory stimuli, neutrophils in the adjacent vasculature initially roll on the blood vessel wall, then stick, and finally transmigrate to the site of insult (1). The initial rolling event involves a class of adhesion proteins termed selectins (P-, E-, and L-selectin), which mediate the interaction between leukocytes and endothelial cells by recognizing specific carbohydrate counterstructures, including sialyll-Lewis x (2–4). P-selectin binds to ~10,000–20,000 copies of a single class of binding site on neutrophils and HL60 cells (4, 5). Studies in a number of laboratories have identified a 220–240-kDa, disulfide-linked homodimeric protein, which appears to specifically bind P-selectin (6, 7). This protein is probably identical to P-selectin glycoprotein ligand-1 (PSGL-1) (8). PSGL-1 is a 220-kDa, disulfide-linked homodimeric sialomucin, which, when expressed in COS cells with the appropriate fusocysltransferase, binds P-selectin in a similar calcium-dependent manner to the receptor on neutrophils. PSGL-1 has a signal peptide sequence of 17 amino acids followed by a 24-amino acid PACE propeptide sequence (8). The mature N terminus of PSGL-1 contains an unusual stretch of 20 amino acids, which is rich in negatively charged aspartate and glutamate residues and which contains three tyrosine residues that meet the consensus sequence for O-sulfation by Golgi sulfotransferase(s) (9). At least one of these tyrosine residues is sulfated as evaluated by site-directed mutagenesis and sulfate labeling experiments.2

PSGL-1 also binds E-selectin. In contrast to P-selectin, however, the requirements for E-selectin recognition are much less rigid. E-selectin binds a number of sialomucin and glycoprotein structures if they co-express the sialyl-Lewis x structure (8, 10). L-selectin binds to a number of different counter-receptors, glycoprotein cell adhesion molecule-1, mucosal addressin cell adhesion molecule-1, and CD34, which, like PSGL-1, are also sialomucins (11). A major question currently unresolved is what determines selectin specificity in the recognition of specific counter-receptor structures. P-, E-, and L-selectin are 60–70% homologous in their N-terminal, lectin motifs, and each similarly recognizes the sialyl-Lewis x and sialyl-Lewis a structures (11). Further, binding of P-selectin to its receptor on neutrophils is 4–5 orders of magnitude more avid than the binding to sialyl-Lewis x (4, 5, 11, 13). While differences in specificity and avidity may in part be accounted for either by the presentation of multiple sialyl-Lewis carbohydrate structures on the receptor mucin core or by subtle differences in carbohydrate structure, it is clear that the protein component of the sialomucin also plays a critical role in selectin interaction (8, 14).

In the present paper, we describe a highly specific metalloproteinase, mocarhagin, which has been purified from the venom of the Mozambiquan spitting cobra, Naja mocambique mocambique. Mocarhagin cleaves a 10-amino acid peptide from the mature N terminus of PSGL-1 and abolishes the ability of PSGL-1 to bind P-selectin. The results are in accord with the negative charge/sulfated tyrosine cluster at the N terminus of
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PSGL-1 being an important determinant of P-selectin recognition in addition to its recognition of carbohydrate structure.

EXPERIMENTAL PROCEDURES

Materials—N. mocambique mocambique venom, disopropyl fluorophosphate (DFP), aprotinin, and pepstatin were purchased from Sigma; Triton X-100 from BDH, Kilsyth, Victoria, Australia; RPMM1 tissue culture medium from Flow Laboratories, Irvine, Scotland; fetal bovine serum from Cytosystems, Castle Hill, N.S.W., Australia; heparin-Sepharose CL-6B, protein A-Sepharose, and Sepharose CL-6B from Pharmacia, Uppsala, Sweden; leupeptin from Auspep, Melbourne, Victoria, Australia. Sodium [125I]iodide and sodium [3H]borohydride were from DuPont NEN. The PSGL-1 peptide, Thr-3 to Glu-17, was synthesized by Chiron, Melbourne, Australia. Synthetic peptides based on the PSGL-1 sequences Glu-1 to Glu-15 and Asp-9 to Arg-23 (Chiron) containing cysteine residues were coupled to keyhole limpet hemocyanin (Sigma) with maleimidobenzenyl-N-hydroxysuccinimide (Pierce), and rabbit antisera were raised and affinity-purified as described previously (15). Non-immune rabbit IgG was also prepared as described previously (5).

Purification of Mocarhagin—The purification and characterization of mocarhagin, a 55-kDa cobra venom metalloproteinase, is to be published elsewhere. Briefly, mocarhagin was purified from crude venom of the snake, N. mocambique mocambique, based on its heparin binding properties. Crude lyophilized venom (0.5 g) was dissolved in water (10 ml) and loaded onto a heparin-Sepharose CL-6B column (1.5 x 40 cm) at 25 ml/h. Following washing with column buffer containing 0.1 M Tris, 0.15 M sodium chloride, pH 7.4 (TS buffer), bound protein was eluted in 0.15 M calcium chloride at pH 7.4. Fractions containing mocarhagin, as assessed by SDS-PAGE, were pooled and concentrated in an Amicon ultrafiltration device and then loaded at 25 ml/h onto a Sepharose CL-6B column (1.5 x 70 cm). Peak eluted fractions were dialyzed against TS buffer. DFP-treated mocarhagin was prepared by treating mocarhagin with 10 mM DFP for 1 h at 22°C, followed by extensive dialysis against the same buffer.

Binding of P-selectin to Neutrophils and HL60 Cells—Binding of [125I]-labeled P-selectin to freshly isolated neutrophils and HL60 cells was performed as described previously in detail (5). To examine the effect of pretreatment of neutrophils or HL60 cells with mocarhagin on P-selectin binding, washed cells (2 x 10^7/ml) in RPMI 1640 containing 1% fetal bovine serum were incubated in the presence or absence of 10 mM EDTA followed by mocarhagin (0.025-100 Mu/ml, final concentration) for 30 min at 22°C. P-selectin binding was then assessed either directly or after the cells were centrifuged, washed twice, and resuspended in RPMI with 3% fetal bovine serum. In some experiments, DFP-treated mocarhagin was inactivated in place by heating mocarhagin. To examine the effect of supernatant from mocarhagin-treated cells on P-selectin binding, HL60 cells at 10^7/ml in 0.1 M Tris, 0.15 M sodium chloride, 0.001 M calcium chloride, pH 7.4, were incubated with mocarhagin (12 ng/ml) for 10 min at 22°C. The supernatant collected following centrifugation at 1,000 x g for 10 min was made 0.1% in BSA and loaded onto a heparin-Sepharose CL-6B column (0.5 x 5 cm) to remove mocarhagin. The flow-through was then tested for its effect on P-selectin binding to HL60 cells. The effect of anti-PSGL-1 peptide antibodies on P-selectin binding was evaluated by incubating washed neutrophils with antibody for 20 min at 22°C prior to the addition of [125I]labeled P-selectin. In addition, immunoprecipitation experiments were performed with mocarhagin (100 ng/ml, final concentration) with or without mocarhagin (12 ng/ml, final concentration) prior to addition to the neutrophils.

Effect of Mocarhagin on Surface-labeled Neutrophils and HL60 Cells—Washed neutrophils or HL60 cells were surface labeled either by lactoperoxidase-catalyzed radiiodination or with sodium [3H]borohydride (16, 17). Labeled cells in 0.01 M Tris, 0.15 M sodium chloride, 1 mM calcium chloride, pH 7.4, were incubated with mocarhagin (12 ng/ml, final concentration) for 10 min at 22°C. The cells were centrifuged at 150 x g for 10 min and washed twice with PBS supplemented with 2% Hepes, 0.15 M sodium chloride, 0.001 M EDTA, pH 7.4. The cells were then lysed with 1% (v/v) Triton X-100 at 4°C for 1 h in the presence of the following protease inhibitors: DFP (0.5 mM), aprotinin (10 ng/ml), pepstatin (10 ng/ml), leupeptin (100 ng/ml), benzamidine (10 ng/ml). The Triton-X 100 soluble fractions separated by centrifugation at 1,000 x g for 10 min and the supernatants from the control and mocarhagin-treated cells were washed with SDS sample buffer and electrophoresed on a 5-15% SDS-polyacrylamide gel under reducing and non-reducing conditions or on a two-dimensional non-reduced/reduced gel as described by Phillips and Agin (18).

Mocarhagin Digestion of Soluble PSGL-1—COS cells were cotransfected with three plasmids encoding soluble PSGL-1 (pE-DspsGL-1.T7), (8), -1,3-L-Fucosyltransferase (PEA.A34FT), and soluble PACE (pE-PAcE-10 nKDa) (19). (PEA.M164 nKDa-labeled COS cells containing sPSGL-1.T7 was digested with 5 ng/ml mocarhagin in TS buffer containing 2 mM calcium chloride, 1 mg/ml BSA for 20 min at 37°C. The ability of sPSGL-1.T7 to bind P-selectin was assessed by precipitation with the P-selectin IgG chimera LEC-Y1 (8) preabsorbed onto protein A-Sepharose beads in TS buffer, 2 mM calcium chloride, 1 mg/ml BSA for 4 h at 4°C. A control experiment was also performed where the LEC-Y1 protein A-Sepharose beads were not treated with mocarhagin and then exhaustively washed prior to presentation of sPSGL-1.T7. For immunoprecipitation analysis of untreated and mocarhagin-treated sPSGL-1.T7, the protease was inactivated by the addition of 10 mM EDTA. The sample was concentrated directly onto Pro-spin (Applied Biosystems, Foster City, CA) and subjected to T-N terminal sequencing on an ABI M476 gas phase protein sequencer. The synthetic peptide corresponding to residues 3-17 of mature PSGL-1 was digested at 0.3 mg/ml in 50 mM Hepes saline buffer, pH 7.4, made 1 mM calcium chloride, and digested with 10 mg/ml mocarhagin for 1 h. After reverse-phase HPLC separation of the reaction products, N-terminal sequence analysis was performed with an Applied Biosystems model 470A protein sequencer.

RESULTS AND DISCUSSION

We have recently identified and purified a novel metalloproteinase, mocarhagin, from the venom of the Mozambican spitting cobra, N. mocambique mocambique. The proteinase requires either calcium ion or zinc ion for activity and is fully inhibited by excess EDTA and high concentrations of DFP. Pretreatment of platelets with mocarhagin abolishes their ability to bind the adhesive ligand, von Willebrand Factor. This is due to proteolysis between Glu-282 and Asp-283, DEGD(T)LYDYYPEE D TEGD, in the α-chain of the platelet GP Ib-V-IX complex, which occurs as the sole detectable cleavage on the intact platelet surface.

In the course of these studies, we observed that mocarhagin was also a potent inhibitor of P-selectin binding to its myeloid receptor, PSGL-1, on neutrophils. Pretreatment of either neutrophils or HL60 cells with mocarhagin profoundly and reproducibly affected the subsequent binding of P-selectin to these treated cells with an apparent IC50 of 0.1 ng/ml. A representative inhibition curve from multiple studies is shown in Fig. 1. Equivalent data were obtained regardless of whether the mocarhagin-treated cells were washed or not washed prior to the addition of P-selectin. Further, inhibition was not reversed by incubation of the treated cells with fresh medium for up to 3 h. Finally, mocarhagin had no apparent effect on the molecular size of P-selectin or on its inherent ability to bind to myeloid cells (data not shown and see Fig. 2). Treatment of mocarhagin with DFP completely blocked its ability to inhibit P-selectin binding even at 100 ng/ml (Fig. 1), a result in accord with proteolysis of the P-selectin receptor. Consistent with this view, the ability of mocarhagin to inhibit subsequent P-selectin binding was divalent cation- and time-dependent. If cells were incubated with 12 ng/ml mocarhagin for 10 s prior to the addition of EDTA and the cells then washed, P-selectin binding was reduced, even with this brief treatment, to 40% of normal (data not shown). Cell surface labeling studies, however, failed to identify a major substrate for mocarhagin on either neutrophils or HL60 cells (data not shown), a finding consistent with
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The exquisite substrate specificity of mocarhagin suggested by the platelet studies. In addition, the concentrated supernatant from mocarhagin-treated cells, after removal of mocarhagin by absorption with heparin-Sepharose CL-6B, did not inhibit binding of P-selectin to HL60 cells, indicating that a functional fragment of the P-selectin receptor was not released by mocarhagin treatment.

PSGL-1 has recently been identified as a functional ligand for P-selectin on HL60 cells (8). A soluble form of PSGL-1 (sPSGL-1T7) expressed in COS cells with an α1,3/1,4-fucosyltransferase also mediates P-selectin binding in a calcium-dependent manner (8). One of the striking features of PSGL-1 is its similarity to the α-chain of platelet GP Ib. Both are sialomucins and each has immediately N-terminal to the mucin core a sequence rich in negatively charged amino acids with three potential sulfated tyrosine residues (8, 20–22). Since mocarhagin cleaves the α-chain of GP Ib within this negative charge/sulfated tyrosine cluster (see above), we speculated that mocarhagin may abrogate P-selectin binding to neutrophils and HL60 cells by cleaving near the N terminus of PSGL-1, a result that would explain the failure to identify a major substrate for mocarhagin. However, there is no evidence that mocarhagin digests PSGL-1 to a soluble fragment of the P-selectin receptor, and the proteolytic activity of mocarhagin is inhibited by heparin, suggesting that the N-terminal epitope for Rb3443 is lost after mocarhagin treatment. This observation is consistent with the sequence of PSGL-1 in P-selectin binding was obtained using a synthetic peptide, TEYELDYDFLPETE, corresponding to residues 3–17 of mature PSGL-1. Mocarhagin digestion of soluble P-selectin glycoprotein ligand, COS-conditioned medium containing [35S]methionine-labeled sPSGL-1T7 was untreated (lanes 1, 3, 5, 6, and 8) or digested with 5 μg/ml mocarhagin (lanes 2, 4, 7, and 9). The samples were either directly electrophoresed (lanes 1 and 2) or precipitated with the P-selectin IgG chimera LECy1 (lanes 3 and 4) or precipitated with LECy1, which was pretreated with mocarhagin (LECy1 + moc, lane 5), or immunoprecipitated with Rb3026 (lanes 6 and 7) or with Rb3443 (lanes 8 and 9).

Confirmation of the critical importance of the N-terminal sequence of PSGL-1 in P-selectin binding was obtained using anti-peptide antibodies. P-selectin binding to neutrophils was inhibited by 80–90% by an affinity-purified polyclonal antibody against residues Gin-1 to Glu-15 of mature PSGL-1 (QATEYELDYDFLPETE) but not by an affinity-purified polyclonal antibody against residues Asp-9 to Arg-23 (DYDFLPETEPEML) (Fig. 3) or by non-immune rabbit IgG (not shown). Inhibition by the anti-peptide antibody against Gin-1 to Glu-15 was completely blocked by the presence of either the Gin-1 to Glu-15 or Asp-9 to Arg-23 peptides (data not shown), indicating that anti-peptide antibody, at least in part, recognizes the sequence, Asp-9 to Glu-15.

Although P-, E-, and L-selectins all recognize similar sialylated carbohydrate structures such as sialyl-Lewis x and many glycoproteins on the surface of myeloid cells contain sialyl-Lewis x, P-selectin appears to be highly specific in its recognition of PSGL-1. The present data suggest that one cause for this specificity is the negative chargesulfated tyrosine cluster at the N terminus of mature PSGL-1. Proteolytic removal of a N-terminal 10-amino acid peptide by mocarhagin abolished P-selectin binding to PSGL-1 even though this sequence (QATEYELDYDFLPETEPEML) is not glycosylated. One explanation for this phenomenon is that removal of this sequence alters the conformational integrity of PSGL-1 such that P-selectin can no longer interact with critical carbohydrate structures associated with the PSGL-1 mucin core. This is unlikely for two reasons. First, an affinity-purified polyclonal antibody against the N-terminal 15 amino acids of mature PSGL-1 also strongly inhibited P-selectin binding to neutrophils. Second, E-selectin also binds to PSGL-1 (8, 25, 26), but, unlike P-selectin, E-selectin binds equally well to mocarhagin-cleaved PSGL-1 (data not shown), suggesting that the carbohydrate recognition structures on PSGL-1 are still inherently accessible. An alternative explanation of the present observations is that P-selectin bind-

**Fig. 1.** Effect of mocarhagin on P-selectin binding to neutrophils. Neutrophils were pretreated for 30 min at room temperature with increasing concentrations of mocarhagin (circles) or with mocarhagin that had been treated with DFP (triangles).

**Fig. 2.** Mocarhagin digestion of soluble P-selectin glycoprotein ligand. COS-conditioned medium containing [35S]methionine-labeled sPSGL-1T7 was untreated (lanes 1, 3, 5, 6, and 8) or digested with 5 μg/ml mocarhagin (lanes 2, 4, 7, and 9). The samples were either directly electrophoresed (lanes 1 and 2) or precipitated with the P-selectin IgG chimera LECy1 (lanes 3 and 4) or precipitated with LECy1, which was pretreated with mocarhagin (LECy1 + moc, lane 5), or immunoprecipitated with Rb3026 (lanes 6 and 7) or with Rb3443 (lanes 8 and 9).
ing to PSGL-1 is bimodal with P-selectin binding not only involving carbohydrate recognition but also the negative charge-sulfated tyrosine cluster. The approximately 4 order of magnitude difference in avidity for P-selectin binding to sialyl-Lewis x versus receptor (11) is strongly suggestive that additional structural determinants are involved in binding of P-selectin to its myeloid receptor. This is supported by the observation that P-selectin binding to myeloid cells not only depends on the N-terminal lectin domain but also involves the adjacent epidermal growth factor-like motif (27). We, and subsequently others, have demonstrated that P-selectin binds to heparin and to a wide variety of other sulfated glycans and polyanionic structures (5, 28). It is tempting to speculate that the N-terminal negative charges/sulfated tyrosine cluster of PSGL-1 represents an equivalent polyanionic recognition site and that the juxtaposition of this sequence with appropriate sialylated carbohydrate structure explains the specificity of P-selectin recognition.

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REFERENCES

1. Butcher, E. C. (1991) Cell 67, 1033–1036.
2. 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.
3. Phillips, M. L., Nudelman, E., Gaeta, F. C. A., Perez, M., Singhal, A. K., Hakomori, S.-I., and Paulson, J. C. (1990) Science 250, 1130–1132.
4. Moore, K. L., Varki, A., and McEver, R. P. (1991) J. Cell Biol. 112, 491–499.
5. Skinner, M. P., Lucas, C. P., Burns, G. F., Chesteman, C. N., and Berndt, M. C. (1991) J. Biol. Chem. 266, 5371–5374.
6. Moore, K. L., Stults, N. L., Diaz, S. M., Smith, D. F., Cummings, R. D., Varki, A., and McEver, R. P. (1992) J. Cell Biol. 118, 445–456.
7. Norgard, K. E., Moore, K. L., Diaz, S. M., Stults, N. L., Ushiyama, S., McEver, R. P., Cummings, R. D., and Varki, A. (1993) J. Biol. Chem. 268, 12764–12774.
8. Sako, D., Chang, X.-J., Barone, K. M., Vachino, G., White, H. M., Shaw, G., Veldman, G. M., Bean, K. M., Ahem, T. J., Furie, B., Cummings, D. A., and Larsen, G. R. (1993) Cell 75, 1179–1186.
9. Huttner, W. B. (1988) Annu. Rev. Physiol. 50, 363–376.
10. Levinovitz, A., Mühlhoff, J., Isenmann, S., and Vestweber, D. (1993) J. Cell Biol. 121, 449–459.
11. McEver, R. P., Varke, K. L., and Cummings, R. D. (1995) J. Biol. Chem. 270, 11025–11028.
12. Varke, A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7390–7397.
13. Nelson, R. M., Dolich, S., Aruffo, A., Cecconi, O., and Bevilacqua, M. P. (1993) J. Clin. Invest. 91, 1157–1166.
14. Larsen, G. R., Sako, D., Ahem, T. J., Shaffer, M., Erban, J., Sajer, S. A., Gibson, R. M., Wagner, D. D., Furie, B. C., and Furie, B. (1992) J. Biol. Chem. 267, 11104–11110.
15. Chong, B. H., Murray, B., Bernett, M. C., Dunlop, L. C., Brighten, T., and Chesterman, C. N. (1994) Blood 83, 1535–1541.
16. Booth, W., Bernett, M. C., and Castaldi, P. A. (1984) J. Clin. Invest. 73, 291–297.
17. Bernett, M. C., and Phillips, D. R. (1981) J. Biol. Chem. 256, 59–65.
18. Phillips, D. R., and Agin, P. P. (1977) J. Biol. Chem. 252, 2121–2126.
19. Wasley, L. C., Rehemtulla, A., Bristol, J. A., and Kaufman, R. J. (1993) J. Biol. Chem. 268, 8436–8440.
20. Lopez, J. A., Chung, D. W., Fujikawa, K., Hagen, F. S., Papayannopoulos, T., and Roth, G. J. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 5615–5619.
21. Tidini, K., Takio, K., Handa, M., and Ruggeri, Z. M. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 5610–5614.
22. Dong, J.-F., Li, C. Q., and López, J. A. (1994) Biochemistry 33, 13946–13953.
23. Lee, N., Wang, W.-C., and Fukuda, M. (1990) J. Biol. Chem. 265, 20476–20487.
24. Fako, M., Spooner, E., Dates, J. E., Dell, A., and Klock, J. C. (1984) J. Biol. Chem. 259, 10925–10935.
25. Moore, K. L., Eaton, S. F., Lyons, D. E., Nudelman, E., Singhal, A. K., Hakomori, S.-I., and Paulson, J. C. (1992) J. Biol. Chem. 267, 23318–23327.
26. Asa, D., Raycroft, L., Ma, L., Aeed, P. A., Kaytes, P. S., Elhammer, A. P., and Geng, J.-G. (1995) J. Biol. Chem. 270, 11662–11670.
27. Kansas, G. S., Saunders, K. B., Ley, K., Zakrzewicz, A., Gibson, R. M., Furie, B. C., Furie, B., and Tedder, T. F. (1994) J. Cell Biol. 124, 609–618.
28. Aruffo, A., Kolanus, W., Walz, G., Fredman, P., and Seed, B. (1991) Cell 67, 35–44.

Fig. 3. Effect of anti-PSGL-1 IgG on P-selectin binding to neutrophils. Dose-response curves for inhibition of specific binding of 125I-P-selectin to neutrophils by polyclonal IgG against synthetic peptide sequences Gln-1 to Glu-15 (circles) and Asp-9 to Arg-23 (squares). Data are representative of at least three experiments with different donor neutrophils.