Two by two: The pairings of P-selectin and P-selectin glycoprotein ligand 1

Guy A. Zimmerman*

Department of Internal Medicine and Program in Human Molecular Biology and Genetics, University of Utah School of Medicine, Salt Lake City, UT 84112

Targeting of circulating leukocytes to specific areas of microbial invasion or tissue injury is critical for host defense and wound repair. If it is defective, syndromes of cellular immune deficiency result; conversely, if targeting is uncontrolled or dysregulated, accumulation of leukocytes may cause inflammatory tissue damage (1–3). In this issue of PNAS, Ramachandran and coworkers (4) report new aspects of the structure-function relationships of two molecules that are critical for precise targeting of myeloid leukocytes and certain other leukocyte subclasses: P-selectin and P-selectin glycoprotein ligand-1 (PSGL-1). P-selectin is a member of the selectin family of adhesion molecules. PSGL-1 on the plasma membranes of neutrophils or monocytes, which are key effector cells of the innate immune system, binds to P-selectin translocated to the surfaces of inflamed endothelial cells or activated platelets (Fig. 1). Forces generated by flowing blood then cause the leukocytes to roll on the surfaces of the endothelial cells or adherent platelets (5, 6). The rolling interaction of myeloid leukocytes on stimulated endothelial cells is the initial cell–cell interaction in the acute inflammatory response. Engagement of PSGL-1 by P-selectin is particularly important at early time points (7, 8), although the rolling component of the multistep process of leukocyte targeting and transmigration to extravascular sites also can be accomplished by other adhesion molecules (5–9).

Specific structural features mediate the pairing of P-selectin and PSGL-1 when they bind to one another (6, 10). The report by Ramachandran *et al.* (4) explores the importance of a second kind of pairing: homodimerization of PSGL-1 and P-selectin. The experiments indicate that this molecular "buddy system" stabilizes rolling of cells and enhances strength of the intercellular tethers when they are subjected to shear. Reversible establishment of bonds in the face of shear forces is a key requirement in the targeting and extravasation of leukocytes from the flowing blood (5, 6, 9).

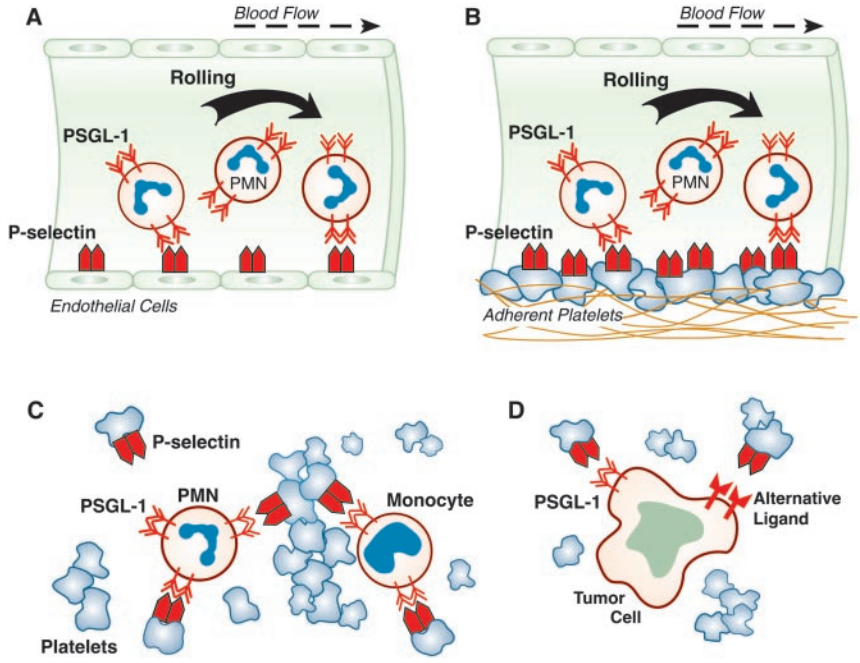

Fig. 1. Cell–cell interactions mediated by P-selectin and PSGL-1. Binding of P-selectin to PSGL-1 mediates rolling of leukocytes on inflamed endothelial cells (*A*) or adherent, activated platelets (*B*). (Neutrophils, or PMNs, are shown but other leukocyte subclasses also roll on P-selectin.) Engagement of PSGL-1 by P-selectin also mediates adhesion of platelets to myeloid leukocytes in suspension, resulting in the formation of mixed cell aggregates (*C*), and is a mechanism for adhesion of some tumor cells to platelets (*D*) and endothelial cells. Adhesive interactions involving P-selectin or PSGL-1 also occur in other circumstances (6, 23).

P-selectin and PSGL-1 are elongate membrane proteins that must be post-translationally altered by addition of glycoconjugates and by other modifications to mediate adhesion (6). P-selectin isolated from human platelets forms dimers and oligimers in solution containing nonionic detergents (11). In addition, crosslinking strategies demonstrate that it exists as homodimers in the plasma membranes of human endothelial cells and platelets (12). Interestingly, a heterodimer involving an unidentified binding partner also may form in the platelet plasma membrane (12), suggesting yet a different sort of pairing. P-selectin homodimers appear to result from interactions between the transmembrane domains of the interacting protein chains (6). PSGL-1 also forms homodimers. One facet of this pairing is covalent linkage by a disulfide bond between membrane-proximate extracellular cysteines in the interacting PSGL-1 chains (13). The second mechanism for homodimeric pairing involves interactions between the transmembrane domains (14). It was previously reported that dimerization is essential for optimal recognition of P-selectin by PSGL-1 (15). However, it is clear that monomeric binding of truncated forms, chimeras, and fragments of the glycoproteins occurs (4, 10, 14). Nevertheless, under static conditions more leukocytes adhere to dimeric P-selectin than to a monomeric form at equivalent densities, suggesting that

See companion article on page 10166.

*E-mail: guy.zimmerman@hmbg.utah.edu.

dimerization confers an adhesive advantage (11).

Although the speculation was that dimerization is important, it was unclear whether it influences rolling or other adhesive events in a flow model. The report by Ramachandran *et al.* (4) argues that it does. Using cell lines transfected with a cDNA for wild-type PSGL-1 or a chimeric form modified to prevent dimerization, they found that cells expressing monomeric or dimeric PSGL-1 tether on immobilized wild-type P-selectin (purified from human platelets) at the same rates. Cells that expressed dimeric PSGL-1 established tethers that were more shear-resistant, however, and had more stable rolling adhesions with less variability in rolling velocity. The findings are important because molecular "braking" that favorably regulates the speed and stability of rolling attachments and enhances exposure of leukocytes to activating signals on or near the endothelial surface—which is required for subsequent firm adhesion and transmigration (1)—may be one of the most important advantages provided by the selectin family and their ligands (5, 16). When Ramachandran *et al.* (4) then substituted monomeric P-selectin for the dimeric form, tether dissociation was again less for cells displaying dimeric PSGL-1 than those that expressed the monomeric chimera. Together, the data suggest that dimerization enhances the formation of second bonds and also facilitates rebinding when the adhesion pair separates, thus stabilizing tethering and rolling. This advantage provided by dimerization may be even more efficient because of clustering PSGL-1 on tips of microvilli, and localization of P-selectin in clusters in microdomains of endothelial cells and, perhaps, platelets (6). Although the experiments (4) were largely done with surrogate cells that could be transfected with the appropriate constructs, comparisons with neutrophils were done and support the conclusion that the findings have physiologic relevance. The stabilizing effects of dimerization were most obvious at low densities of P-selectin and PSGL-1 in the face of increasing shear stresses. This observation also has physiologic relevance because the surface densities of P-selectin on stimulated endothelial cells and activated platelets vary significantly (6). All in all, the new experiments exploring the dimerization of P-selectin and PSGL-1 suggest that these molecular pairings have conferred evolutionary and biologic advantages for a specialized adhesive interaction (17) critical for inflammation and vascular repair: leukocyte rolling.

Rolling interactions mediated by selectins and their ligands have received a great deal of attention because of their temporal place in targeting of leukocytes to tissues and their requisite contributions to many inflammatory responses. It shouldn't be forgotten, however, that selectins mediate other adhesive events besides rolling of leukocytes on endothelial cells on platelets immobilized at sites of vascular injury. Two additional cell–cell interactions mediated by the P-selectin/PSGL-1 binding pair are illustrated in Fig. 1. In the first, P-selectin on activated platelets in suspension binds to PSGL-1 on neutrophils or monocytes, contributing to the formation of mixed cell aggregates (6, 18). Platelet adhesion to leukocytes occurs both *in vitro* and *in vivo* and the attachments can be stable over many hours, in contrast to transient rolling interactions. Adhesion via P-selectin and PSGL-1 may contribute to the "piggybacking" of platelets into inflamed tissues attached to transmigrating leukocytes, which occurs in some situations (19), and likely contributes to intravascular leukocyte sequestration in injured or intensely inflamed vessels. One consequence of binding of P-selectin to PSGL-1 on human monocytes and neutrophils is outside-in signaling, leading to modulation of integrin function, activation of intracellular kinase cascades, and signaling to checkpoints that regulate gene expression (18, 20–23). Dimerization of PSGL-1 and/or P-selectin may clearly influence these signaling events, in addition to mediating intercellular tethering. In a second example (Fig. 1), P-selectin on platelets or endothelial cells mediates binding to some tumor cells, providing mechanisms for rolling, localization, metastasis, and neoplastic progression (23, 24). Again, dimerization may critically influence the ability of the selectin and its ligand to accomplish cell–cell attachment and intercellular signaling. A caveat here is that P-selectin has other binding partners that it pairs with on both primary and neoplastic cells (5, 6, 23), in addition to PSGL-1, and there may be heterogeneity in the factors that influence recognition of these alternative ligands. Characterization of optimal determinants for one adhesive interaction such as rolling (4) may yield insights into others (Fig. 1), and will be important as compounds are evaluated for their potential to interrupt pathologic pairings of P-selectin, PSGL-1, and other selectins and selectin ligands (25).

1. Zimmerman, G. A., McIntyre, T. M. & Prescott, S. M. (1996) *J. Clin. Invest.* **98,** 1699–1702.
2. Harris, E. S., Shigeoka, A. O., Li, W., Adams, R. H., Prescott, S. M., McIntyre, T. M., Zimmerman, G. A. & Lorant, D. E. (2001) *Blood* **97,** 767–776.
3. Luhn, K., Wild, M. K., Eckhardt, M., Gerardy-Schahn, R. & Vestweber, D. (2001) *Nat. Genet.* **28,** 69–72.
4. Ramachandran, V., Yago, T., Epperson, T. K., Kobzdej, M. M. A., Nollert, M. U., Cummings, R. D., Zhu, C. & McEver, R. P. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 10166–10171. (First Published July 31, 2001; 10.1073/pnas.171248098)
5. Ley, K. (1999) in *Vascular Adhesion Molecules and Inflammation*, ed. Pearson, J. (Birkhauser, Basel), pp. 11–37.
6. McEver, R. P. (2001) *Hemostasis Thrombosis*, in press.
7. Mayadas, T. N., Johnson, R. C., Rayburn, H., Hynes, R. O. & Wagner, D. D. (1993) *Cell* **74,** 541–554.
8. Yang, J., Hirata, T., Croce, K., Merrill-Skoloff, G., Tchernychev, B., Williams, E., Flaumenhaft, R., Furie, B. C. & Furie, B. (1999) *J. Exp. Med.* **190,** 1769–1782.
9. Siegelman, M. (2001) *J. Clin. Invest.* **107,** 159–160.
10. Somers, W. S., Tang, J., Shaw, G. D. & Camphausen, R. T. (2000) *Cell* **103,** 467–479.
11. Ushiyama, S., Laue, T. M., Moore, K. L., Erickson, H. P. & McEver, R. P. (1993) *J. Biol. Chem.* **268,** 15229–15237.
12. Barkalow, F. J., Barkalow, K. L. & Mayadas, T. N. (2000) *Blood* **96,** 3070–3077.
13. Moore, K. L., Stults, N. L., Diaz, S., Smith, D. F., Cummings, R. D., Varki, A. & McEver, R. P. (1992) *J. Cell Biol.* **118,** 445–456.
14. Epperson, T. K., Patel, K. D., McEver, R. P. & Cummings, R. D. (2000) *J. Biol. Chem.* **275,** 7839–7853.
15. Snapp, K. R., Craig, R., Herron, M., Nelson, R. D., Stoolman, L. M. & Kansas, G. S. (1998) *J. Cell Biol.* **142,** 263–270.
16. Chen, S. & Springer, T. A. (1999) *J. Cell Biol.* **144,** 185–200.
17. Hynes, R. O. & Zhao, Q. (2000) *J. Cell Biol.* **150,** F89–F96.
18. Weyrich, A. S., Elstad, M. R., McEver, R. P., McIntyre, T. M., Moore, K. L., Morrissey, J. H., Prescott, S. M. & Zimmerman, G. A. (1996) *J. Clin. Invest.* **97,** 1525–1534.
19. Issekutz, A. C., Ripley, M. & Jackson, J. R. (1983) *Lab. Invest.* **49,** 716–724.
20. Lorant, D. E., Patel, K. D., McIntyre, T. M., McEver, R. P., Prescott, S. M. & Zimmerman, G. A. (1991) *J. Cell Biol.* **115,** 223–234.
21. Hidari, K. I., Weyrich, A. S., Zimmerman, G. A. & McEver, R. P. (1997) *J. Biol. Chem.* **272,** 28750–28756.
22. Mahoney, T. S., Weyrich, A. S., Dixon, D. A., McIntyre, T., Prescott, S. M. & Zimmerman, G. A. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 10284–10289. (First Published August 21, 2001; 10.1073/pnas.181201398)
23. Vestweber, D. & Blanks, J. E. (1999) *Physiol. Rev.* **79,** 181–213.
24. Kim, Y. J., Borsig, L., Varki, N. M. & Varki, A. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 9325–9330.
25. Leppanen, A., Mehta, P., Ouyang, Y. B., Ju, T., Helin, J., Moore, K. L., van Die, I., Canfield, W. M., McEver, R. P. & Cummings, R. D. (1999) *J. Biol. Chem.* **274,** 24838–24848.