Editorial

Platelets, Endothelial Cells, Inflammatory Chemokines, and Restenosis

Complex Signaling in the Vascular Play Book

Andrew S. Weyrich, PhD; Stephen M. Prescott, MD; Guy A. Zimmerman, MD

Adhesive and signaling interactions between endothelial cells and leukocytes are key mechanisms that mediate both targeting of the blood cells to specific sites and information transfer that results in change in their phenotypes and function.[1] These events are beneficial in defense against microbes and wound surveillance and repair; however, in dysregulated inflammatory conditions such as atherosclerosis and its complications, including acute coronary syndromes and restenosis after interventional procedures, accumulation and activation of leukocytes can injure the host. The cell–cell interactions and signaling mechanisms are complex in the game of inflammation, and there are a number of players. In addition to endothelial cells and leukocytes, platelets contribute to leukocyte accumulation and to changes in their behavior in both physiological and pathological conditions,[2,3,4] emphasizing the intimate relationship between the thrombotic and inflammatory systems.

See p 1523

One mechanism involves direct adhesion of activated platelets to monocytes, neutrophils, or other leukocyte subclasses and binding of chemokines or lipid signaling molecules that are released from the platelets or displayed in a juxtacrine fashion on their surfaces (see below), to receptors on the leukocyte plasma membrane.[3,5] In this issue of *Circulation*, Schober and co-workers[6] report a different play in the inflammatory repertoire: platelets interacting with inflamed endothelial cells donate the CC chemokine regulated upon activation normal T cell expressed presumed secreted (RANTES) to the endothelial surface, where it acts as a cell-associated signal for adhesion of monocytes. A number of the observations were made with cultured endothelial monolayers, incubated under conditions of stasis or flow, and isolated leukocytes; a caveat is that these experiments were done with proprietary endothelial cells and a monocytic cell line rather than primary leukocytes. In addition, however, other strategies added to the findings. Receptor blocking experiments indicated that RANTES signaling contributes to intimal hyperplasia and macrophage accumulation in an in vivo model of restenosis in atherosclerosis-prone apolipoprotein (apoE)-deficient mice.[6] Interestingly, the transfer of RANTES from platelets to the endothelial plasma membrane seems not to require tight adhesion between these two cells. Instead, it appears to be a "give and go" mechanism that involves transient interaction of the two cells that is nonetheless sufficient for delivery of the chemokine. In vivo experiments using P-selectin–deficient mice crossed to apoE-deficient animals in parallel with in vitro assays indicated that P-selectin is required for monocyte and macrophage accumulation and intimal hyperplasia.[6] This is not unexpected, since P-selectin mediates adhesion, signaling, and gene expression when the ligand for P-selectin on monocytes and other leukocytes, P-selectin glycoprotein ligand-1 (PSGL-1), is engaged by P-selectin that is displayed by activated endothelial cells or platelets.[1–5,7] In addition, P-selectin is critical for intimal hyperplasia in other murine models of vascular injury.[3,6,8] The surprise is that in the experiments reported by Schober et al,[6] platelet P-selectin was required for transfer of RANTES to the endothelial surface, whereas P-selectin on endothelial cells was not involved. In this vascular game involving three franchise players — endothelial cells, platelets and leukocytes — the "give and go" route is complex indeed when the RANTES signal is called.

Signaling of leukocytes by factors localized on the surfaces of endothelial cells has been known for some time. The first mechanism to be reported was binding of platelet-activating factor (PAF), a phospholipid, to its receptor on target neutrophils (PMNs) while associated with the plasma membranes of endothelial cells. This signaling event triggers spatially-regulated activation of the PMNs and a variety of responses, including activation of β_2 integrins, which mediates tight adhesion of the leukocytes to the endothelial surface.[1,4] PAF is rapidly synthesized by endothelial cells stimulated with thrombin or other agonists, and is translocated to the endothelial plasma membrane but retained there rather than being released into solution. Thus, this is a juxtacrine mechanism in which a signaling molecule is recognized by its receptor on a "target" cell and activates it, while remaining associated with the cell of origin.[1,4] In this case the signaling molecule is PAF, and the signaling cell is an inflamed or injured endothelial cell. PAF can also signal PMNs in a juxtacrine fashion when it is synthesized and displayed on the surfaces of adherent activated platelets in models of thrombosis and its inflammatory sequellae.[4] When PAF signals PMNs in a juxtacrine fashion at the surfaces of stimulated endothelial cells or platelets, it acts in cooperation with P-selectin, which

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

From the Department of Internal Medicine (A.S.W., S.M.P., G.A.Z.), The Huntsman Cancer Institute (S.M.P.), and The Program in Human Molecular Biology and Genetics (A.S.W., G.A.Z.), The University of Utah, Salt Lake City.

Correspondence to Guy Zimmerman, Program in Human Molecular Biology and Genetics, Eccles Institute of Human Genetics, 15 N 2030 East, Bldg. 533, Rm. 4220, University of Utah, Salt Lake City, UT 84112. E-mail guy.zimmerman@hmbg.utah.edu

(*Circulation* 2002;106:1433-1435.)

***Circulation* is available at http://www.circulationaha.org**
DOI: 10.1161/01.CIR.0000033634.60453.22

is simultaneously displayed on these cells and is required for tethering of the target PMN.[1,4]

In addition to PAF, chemokines can engage their receptors and send outside-in signals while displayed on cellular surfaces. The earliest examples involved the C-X-C chemokine interleukin-8, and were again mechanisms for spatially-localized signaling and activation of PMNs.[1,9] Subsequently, RANTES was shown to have the capacity to signal monocytes, which bear the CCR1 and CCR5 receptors that recognize it, when immobilized on the surfaces of endothelial cells and to promote monocyte migration by haptotaxis when localized in this fashion.[10] In this study, in situ hybridization failed to detect mRNA for RANTES in inflamed endothelial cells in tissue biopsies, however, suggesting that the chemokine was released by another cell type and was then bound by endothelium. The article by Schober et al[6] in this issue, as well as an earlier report from this group, indicate that platelets can donate RANTES to endothelial cells for presentation to monocytes and that it can activate the leukocytes under conditions of flow while displayed at the endothelial surface.[6] Thus, this is different from the juxtacrine mechanism outlined above because the signaling molecule, RANTES, is passed from one blood cell — the platelet — to the endothelial surface to be used to activate another intravascular cell type — the monocyte. Platelets are known to store RANTES in intracellular granules in the basal state and to release it when activated.[2,11] However, the pass from platelets to endothelial cells in the current report is not a "long bomb" in which RANTES circulates over distance but rather a shuttle transfer that requires transient adhesion involving P-selectin.[6] In addition, the endothelial cells must be appropriately conditioned to receive the inflammatory chemokine: deposition of RANTES on cultured endothelial cells required prior stimulation of the endothelial monolayers with interleukin 1β (IL-1β).

While there are several potential sources of IL-1β in the inflammatory and atherosclerotic milieu, this is another pass that platelets can make. Human platelets activated by thrombin or PAF release IL-1β in microvesicles and soluble form in sufficient quantities to induce inflammatory gene expression in endothelial cells.[12–14] This involves a previously-unrecognized ability of activated platelets to synthesize IL-1β and certain other proteins from constitutive messenger RNAs.[14] The change in the surfaces of endothelial cells stimulated by IL-1β that allows them to capture and present RANTES to monocytes is not clear.[6] The CC chemokine receptors that recognize RANTES were not present on IL-1–stimulated endothelial cells, excluding one possibility. In addition, there is a more likely group of candidate molecules. RANTES and several other chemokines are localized to cell surfaces and immobilized on extracellular matrix by binding to glycosaminoglycans via heparin recognition motifs,[1,9,15] a feature of the endothelial surface that may be altered by cytokine activation.

Although signaling of target leukocytes by chemokines and PAF presented at endothelial surfaces is of considerable interest in the context of mechanisms of restenosis, as well as for trafficking and accumulation of monocytes earlier in atherogenesis and for neutrophil activation in acute coronary syndromes, these are not the only pathological situations in which this mechanism may be important. A similar mechanism has been proposed in other syndromes of inflammation and vascular pathology,[4,6,10] and chemokine presentation at endothelial surfaces may mediate tumor cell metastasis.[16] There are important corollaries to this mode of intercellular signaling. One is that it may be difficult to detect it in vivo, and the concentrations of chemokines or other signaling molecules in solution may have little or no relationship to their actions when they are signaling while associated with cellular surfaces. Another is that activation of target cells by signaling molecules localized to cell surfaces may be more difficult to inhibit than when they are presented to their receptors in solution.[4]

In the study of platelet–endothelial interactions,[6] the mechanisms of transfer of RANTES and the identity of its binding sites on the endothelial surface are not the only questions that emerge from watching the "give and go" replays. The experiments indicated to the authors that transient adhesion between platelets and endothelial cells that is dependent on platelet P-selectin allows sufficient contact for deposition of RANTES, and that this is facilitated by flow. The nature of the binding partner for P-selectin on the endothelial surface was not defined and blocking experiments excluded the best characterized ligand, PSGL-1, as being important in the RANTES transfer. PSGL-1 is dominantly expressed by myeloid leukocytes, so this is not a surprise, although it has recently been reported to be present on platelets at low levels.[3] However, this leaves the identity of alternative endothelial ligands that recognize P-selectin and facilitate transfer of RANTES unknown. Evidence that platelets specifically tether to the surfaces of endothelial cells, even transiently, has been reported only relatively recently in contrast to their well-characterized binding to sub-endothelial matrix proteins exposed by wounding or interventional manipulation.[3,5,8] Endothelial cells have several mechanisms that inhibit platelet adhesiveness, including the ability to synthesize prostacyclin, prostaglandin E_2 and nitric oxide, and conversely do not have known constitutive adhesive systems for platelets on their surfaces. Thus, stimulation of endothelial cells with IL-1β and their conversion to a prothrombotic and proinflammatory phenotype is likely to again be a key requirement, regardless of the identity of the binding site(s) that recognize platelet P-selectin. Other questions also come to mind. In the P-selectin/apoE –deficient animal model used in the study,[6] it seems possible that uptake of RANTES into alpha granules of maturing platelets may have been reduced or eliminated because of interruption of key cell-cell interactions dependent on P-selectin at the megakaryocyte stage. While this would be an unexpected and cryptic explanation for the in vivo findings, it would be of interest for this and similar models and could be examined by assaying RANTES in platelets from P-selectin knockout mice. Other unknowns in the study include the requirements and roles of chemokines that are endogenously synthesized by inflamed endothelial cells, in addition to RANTES transferred from platelets, in promoting monocyte accumulation and macrophage differentiation in postinterventional restenosis. Treatment of apoE-deficient mice with a competitive antagonist of the RANTES

receptor reduced neointimal macrophage infiltration and restenotic lesion formation by 40%, suggesting that other signaling molecules and intercellular interactions are also important. Furthermore additional chemokines, including monocyte chemotactic protein 1 (MCP-1), have been implicated in studies of human and experimental restenosis.[17,18]

A growing body of evidence indicates that inflammation is a critical feature of postintervention restenosis in animal models[6,8,18] and in vessels of human patients subjected to angioplasty and stenting.[17,19] This involves more than targeting of monocytes to restenotic sites, which RANTES appears to facilitate in the "give and go" play between platelets and endothelium. There are also changes in phenotype, function, and gene expression in adherent monocytes, both acutely and as they differentiate into macrophages. These inflammatory responses, too, are modulated by RANTES and P-selectin, underscoring that these factors influence many cellular events in monocytes and macrophages in addition to their localization. A prime opportunity for signaling of new gene expression occurs when platelets adhere directly to monocytes or other leukocytes at sites of vascular injury.[2,4,5,7,8] Interestingly, specific gene products expressed by adherent activated monocytes are under differential transcriptional and posttranscriptional control,[2,4,5,7] including a subset of mRNAs that is regulated by mammalian target of rapamycin (mTOR).[7] Thus, interruption of inflammatory signaling and gene expression, in addition to inhibiting smooth muscle proliferation and migration, may be a component of the beneficial effect of rapamycin, a new player in the defensive armamentarium against restenosis.[20] This emphasizes the importance of knowing the diverse signaling mechanisms in the vascular play book for devising new therapies and understanding their mechanisms of action. The contributions of RANTES to inflammatory signaling of gene expression in target monocytes when it is displayed on the surfaces of endothelial cells after the "give and go" play[6] compared with its direct pass off from platelets that are adherent to monocytes via P-selectin and PSGL-1[2] remains to be explored. Presentation of RANTES at the endothelial surface may occur relatively late after interventional injury to the vessel,[6] whereas interaction of platelets and monocytes can occur quite early[8]. Both the timing and the molecular context of the signaling event (ie, the other chemokines and signaling factors presented to the monocyte in sequence and parallel to RANTES) will likely change the outcome of the play and the patterns of genes and other responses that are triggered.

References

1. Zimmerman GA, McIntyre TM, Prescott SM. Adhesion and signaling in vascular cell–cell interactions. *J Clin Invest*. 1996;98:1699–1702.
2. Weyrich AS, Elstad MR, McEver RP, et al. Activated platelets signal chemokine synthesis by human monocytes. *J Clin Invest*. 1996;97: 1525–1534.
3. McEver RP. Adhesive interactions of leukocytes, platelets, and the vessel wall during hemostasis and inflammation. *Thromb Haemost*. 2001;86: 746–756.
4. Dixon DA, McIntyre T, Prescott SM, et al. Molecular mechanisms of juxtacrine cell signaling in microvascular responses and inflammation. In: G. Schmid-Schonbein, DN Granger, eds. *Molecular Mechanisms of Microvascular Disorders*. Paris, France: Springer-Verlag, 2002. In press.
5. Galt SW, Lindemann SL, Medd D, et al. Differential regulation of matrix mellatoproteinase-9 by monocytes adherent to collagen and platelets. *Cir Res*. 2001;89:509–516.
6. Schober A, Manka D, von Hundelshausen BS, et al. Deposition of platelet RANTES triggering monocyte recruitment requires P-selectin and is involved in neointimal formation after arterial injury. *Circulation*. 2002; 106:1523–1529.
7. Mahoney TS, Weyrich AS, Dixon DA, et al. Cell adhesion regulates gene expression at translational checkpoints in human myeloid leukocytes. *Proc Natl Acad Sci U S A*. 2001;98:10284–10289.
8. Smyth SS, Reis ED, Zhang W, et al. β3-integrin–deficient mice but not P-selectin–deficient mice develop intimal hyperplasia after vascular injury: correlation with leukocyte recruitment to adherent platelets 1 hour after injury. *Circulation*. 2001;103:2501–2507.
9. Rot A. Endothelial cell binding of NAP-1/IL-8: role in neutrophil emigration. *Immunol Today*. 1992;13:291–294.
10. Wiedermann CJ, Kowald E, Reinisch N, et al. Monocyte haptotaxis induced by RANTES chemokine. *Current Biology*. 1993;3:735–739.
11. Kameyoshi Y, Dorschner A, Mallet AI, et al. Cytokine RANTES released by thrombin-stimulated platelets is a potent attractant for human eosinophils. *J Exp Med*. 1992;176:587–592.
12. Hawrylowicz CM, Santoro SA, Platt FM, et al. Activated platelets express IL-1 activity. *J Immunol*. 1989;143:4015–4018.
13. Loppnow H, Bil R, Hirt S, et al. Platelet-derived interleukin-1 induces cytokine production, but not proliferation of human vascular smooth muscle cells. *Blood*. 1998;91:134–141.
14. Lindemann S, Tolley ND, Dixon DA, et al. Activated platelets mediate inflammatory signaling by regulated interleukin 1beta synthesis. *J Cell Biol*. 2001;154:485–490.
15. Proudfoot AE, Fritchley S, Borlat F, et al. The BBXB motif of RANTES is the principal site for heparin binding and controls receptor selectivity. *J Biol Chem*. 2001;276:10620–10626.
16. Murphy PM. Chemokines and the molecular basis of cancer metastasis. *N Engl J Med*. 2001;345:833–835.
17. Cipollone F, Marini M, Fazia M, et al. Circulating levels of monocyte chemoattractant protein-1 in patients with restenosis after coronary angioplasty. *Arterioscler Thromb Vasc Biol*. 2001;21:327–334.
18. Schmidt AM, Stern DM. Chemokines on the rise: MCP-1 and restenosis. *Arterioscler Thromb Vasc Biol*. 2001;21:297–299.
19. Farb A, Weber DK, Kolodgie FD, et al. Morphological predictors of restenosis after coronary stenting in humans. *Circulation*. 2002;105: 2974–2980.
20. Morice MC, Serruys PW, Sousa JE, et al. A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. *N Engl J Med*,. 2002;346:1773–1780.

KEY WORDS: Editorials ■ endothelium ■ platelets ■ leukocytes ■ inflammation

Platelets, Endothelial Cells, Inflammatory Chemokines, and Restenosis: Complex Signaling in the Vascular Play Book

Andrew S. Weyrich, Stephen M. Prescott and Guy A. Zimmerman

Circulation. 2002;106:1433-1435
doi: 10.1161/01.CIR.0000033634.60453.22
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539