Perspectives Series: Cell Adhesion in Vascular Biology

Adhesion and Signaling in Vascular Cell–Cell Interactions

Guy A. Zimmerman,*‖ Thomas M. McIntyre,*‖¶ and Stephen M. Prescott‡‖§

**The Nora Eccles Harrison Cardiovascular Research and Training Institute, ‡The Eccles Program in Human Molecular Biology and Genetics, and §Department of Biochemistry, ‖Department of Internal Medicine and ¶Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, Utah 84112*

Cellular interactions are critical events in vascular biology. These include adhesion and the transfer of information between endothelial cells and blood cells, in platelet aggregation and interactions between leukocytes, and in contacts between cells of the vessel wall. Specific molecular mechanisms govern these cell–cell interactions and, by doing so, regulate events in homeostatic inflammation and coagulation, orchestrate wound repair, and influence vasomotor responses. In contrast dysregulated, or unregulated, cell–cell interactions lead to pathologic syndromes: leukocytes that normally protect against invading bacteria go awry in ischemia-reperfusion disorders and acute respiratory distress syndrome, hemostasis becomes occlusive thrombosis, and atherosclerosis occurs where there should be orderly repair of a wounded vessel. Cell–cell interactions in these and other maladies are targets for therapeutic intervention. Whether such strategies can be successfully applied without unacceptably interrupting normal functions is largely unknown.

The molecular mechanisms that operate in vascular cell interactions are also relevant to other important processes, including organ development, wound repair, and neoplasia and metastasis. Because the paradigms are fundamental, what has been learned in the vascular system has given useful insights into these processes. One important lesson is that adhesion between cells is required for targeting them to specific sites as well as for anchoring them in place. A second lesson is that adhesion and intercellular signaling are intimately related events (1). A corollary is that coordination of the two processes can be accomplished by a variety of mechanisms. Other perspectives in this series will deal with individual adhesion molecules and with many particular cellular interactions. Here we will focus on the relationship between adhesion and signaling.

Cellular interactions go on over distance or can occur in close proximity. Endocrine mechanisms have evolved to allow signaling between cells in different anatomic locations. Paracrine mechanisms shorten the distance a signaling molecule must traverse before it reaches its receptor on a target cell and activates its intracellular transduction cascades. Prostacyclin and nitric oxide are topical examples of paracrine signaling molecules that act in the vasculature. Making the intercellular signaling adhesion-dependent refines the spatial precision to an even greater degree, establishing a powerful control mechanism. The simplest mechanism is when an adhesion molecule on one cell binds to a counterligand on another, both tethering the cells together and transmitting a signal that alters the function of the second cell. This has been termed juxtacrine signaling (2) (see below). There is considerable evidence that adhesion factors that operate in the vascular system (including selectins, integrins, members of the intercellular adhesion molecule [ICAM] family and others in the immunoglobulin superfamily, and counterligands for these molecules) interface directly or indirectly with intracellular transduction systems. Thus, these molecules have the potential to mediate both tethering and intercellular signaling. Outside-in signaling by integrins is the most well-known example, although this has often been studied in cell–matrix (3) rather than cell–cell interactions. In some cases signals may be transmitted by both partners of a ligand pair, mediating bidirectional cross talk between cells. Intercellular adhesion and signaling mediated by one adhesion factor binding to its counterligand is elegantly simple and parsimonious. However, in many cases adhesion and signaling by a single molecule and its ligand may have limitations or drawbacks: such a mechanism lacks the checkpoints and redundancy that combinations of molecules provide. Another lesson that has been learned in the vascular system is that combinatorial display of tethering and signaling factors is a mechanism for achieving adhesion-dependent signaling (1).

The interactions between endothelial cells and leukocytes have been particularly informative with respect to adhesion and signaling and have demonstrated intricate combinatorial mechanisms. We and others have studied the adhesion of neutrophils (PMNs) to endothelial cells and the signaling events that are involved (1) to determine how this class of leukocytes is attracted to the proper site in the vasculature, how they are activated, and what safety features allow these events to occur without concurrent damage to the vessel wall. Observations by many early investigators, often using intravital microscopy (4), predicted that precise molecular mechanisms govern this cell–cell interaction. One such feature is rapid targeting of PMNs only to endothelium adjacent to sites of tissue infection or damage, and not to upstream or downstream sites. In addition,

Address correspondence to Guy A. Zimmerman, M.D., University of Utah, CVRTI, Building 500, Salt Lake City, UT 84112. Phone: 801-581-8183; FAX: 801-581-3128; E-mail: guy_zimmerman@gator mail.cvrti.utah.edu

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there is localized signaling of adherent PMNs (identified in early studies by development of polarized morphology, which requires cellular activation), and the adhesion of the PMNs to the endothelial cells is reversible, allowing the leukocytes to emigrate to extravascular sites.

Using cultured human endothelial cells stimulated with thrombin, histamine, or certain other agonists, and isolated PMNs, we identified a mechanism that incorporates each of the characteristics of neutrophil targeting and activation that were described in vivo. This involves two adhesive interactions mediated by factors of different classes. A loose adhesive event is mediated by P-selectin, which is translocated to the plasma membranes of the stimulated endothelial cells. This is an essential point: the surfaces of the endothelial cells change in a way that initiates the adhesive interaction, thus establishing spatial specificity (5). When endothelial cells are stimulated by different inflammatory agonists, other members of the selectin family are called into play including E-selectin expressed on stimulated endothelial cells and L-selectin on PMNs, which recognizes a counterligand that is not present on resting endothelium. Selectin bonds are rapidly established and reversed, are resistant to shear, and, importantly, do not require leukocyte activation (6). Thus, they are well-suited for an initial tethering event and for in vivo adhesion of PMNs in flowing blood. When PMNs interact with endothelial cells in postcapillary venules in vivo, the initial loose step mediated by selectins is manifested as rolling of PMNs along the endothelial surface (1, 6). However, in flowing blood, and in the in vitro model, a second tethering interaction is required for the PMNs to tightly adhere. In contrast to the initial selectin step, this tight adhesion does require signaling and activation of the neutrophil. The cellular activation leads to a functional change in integrins of the β_2 family on the PMN plasma membranes (a process termed "inside-out" signaling), making the integrins competent to bind to counterligands on the endothelial surface and strengthening the initial tethering. Thus, there is adhesion mediated by a selectin, a signaling step (also called "triggering"), and a second amplifying adhesive interaction mediated by β_2 integrins that depends on signaling.

The critical contributions of the adhesion molecules in endothelial interactions with PMNs have been established in isolated cell systems, in experimental animals manipulated by genetic modification or by administration of blocking antibodies, and by analysis of cells from subjects with leukocyte adhesion deficiency types I and II, which are deficient in β_2 integrins and selectin ligands, respectively. The signaling component is also critical: if the activation-dependent step mediated by the β_2 integrins does not occur the PMNs cannot adhere tightly and cannot transmigrate to extravascular sites. In the in vitro model outlined above, we found that antibodies against P-selectin inhibited both adhesion and signaling (7, 8). However, P-selectin alone in model membranes or expressed on transfected cells did not directly induce inside-out signaling of β_2 integrins or other activation responses (8). Thus, another factor delivers the signal, although adhesion via P-selectin is required. This illustrates a combinatorial editing or fail-safe mechanism: engagement and tethering of the target cell by an adhesion molecule is required for the signaling action of another factor.

Neutrophil activation occurs in response to signaling molecules that are recognized by receptors on their plasma membranes, including members of the serpentine G-protein–linked receptor class, receptors that recognize TNFα or GM-CSF, and others (9). Traditionally these have been called chemotactic factors and were thought to be supplied by cells outside of the vascular system, that is, at sites relatively distant from the initial contact between endothelial cells and PMNs. However, an important point is that inflamed endothelial cells express signaling factors for PMNs. This is a powerful mechanism for spatially localizing the activation response of the target leukocytes and one that potentially eliminates the need for release of signaling factors into solution, which can have deleterious consequences (10). In the in vitro model that we have used for illustration, the signaling molecule is platelet-activating factor (PAF),[1] a phospholipid. PAF is rapidly synthesized by the stimulated endothelial cells, but its expression on their surfaces is transient; thus, there are mechanisms that control its signaling action (11). PAF induces inside-out signaling of β_2 integrins on the PMN and other activation responses, and blocking the serpentine receptor for PAF on the PMN inhibits these events at the endothelial surface (7, 12). In addition, purified PAF induces the relevant signaling events and does so when PMNs are tethered to purified P-selectin (8). Engagement of the ligand for P-selectin on PMNs appears to facilitate inside-out signaling of β_2 integrins (7). Thus, P-selectin and PAF act in a complementary fashion. Such coordinate action of tethering and signaling factors is likely to be a general mechanism of adhesion-dependent signaling in the vascular system and elsewhere. Finally, PAF can provide the signal that modifies the bonds that initially tether the PMN to the surface of inflamed endothelial cells, a process that is likely required for effective transmigration (13). How adhesive bonds are modified and broken in other cell types that become tightly tethered in one place in the vascular system, but then must move to another, is a topic for exploration.

PAF was the first proadhesive signaling molecule for neutrophils to be identified as a product of stimulated endothelial cells (11). More recently, chemokines of the C-X-C family have been found to be products of stimulated endothelial cells, including IL-8 (14) and ENA-78 (Imaizumi, T., K.H. Albertine, D.L. Jicha, T.M. McIntyre, S.M. Prescott, and G.A. Zimmerman, manuscript submitted for publication), which are recognized by related serpentine receptors on PMNs and induce β_2 integrin–dependent adhesiveness. Chemokines of the C-C class are also synthesized by stimulated endothelial cells. The most carefully studied example to date is monocyte chemotactic protein-1, which activates monocytes rather than neutrophils (9). Chemokines of both the C-X-C and C-C families are synthesized by endothelial cells depending on the inflammatory agonist, the time after stimulation, and other factors. How their genes are controlled in relationship to one another is largely unknown. As with adhesion molecules, the regulated expression of different signaling molecules (PAF, C-X-C chemokines, C-C chemokines) by endothelial cells in different temporal patterns, in response to different agonists and, potentially, in different vascular beds provides a mechanism for differential activation of specific classes of leukocytes (1).

PAF signals neutrophils while remaining associated with the endothelial plasma membrane (12). Thus, it acts in a juxtacrine fashion (2, 7, 10). Juxtacrine signaling adds another level of precision to spatially localized information transfer between

1. *Abbreviations used in this paper:* PAF, platelet-activating factor; PSGL-1, P-selectin glycoprotein ligand-1.

cells and is particularly well-suited for inflammatory and vascular interactions, where tightly localized cellular activation is frequently a requirement. It has also been identified in other highly regulated biologic systems where precise control of information transfer between cells is critical, including the *Drosophila* eye, the mouse bone marrow, *Xenopus* embryos, and others (2, 10). In endothelial cell interactions with leukocytes, E-selectin may act as a juxtacrine signaling molecule, and IL-8 and certain other chemokines may signal while localized at the endothelial surface, by virtue of binding to membrane glycosaminoglycans; the latter is a variation on the original juxtacrine mechanism (for review see reference 10). There is also evidence that endothelial cells, in turn, can be signaled in a juxtacrine fashion by adherent leukocytes presenting membrane-bound IL-1 or TNFα (15, 16). How and where juxtacrine signaling occurs between other cells in the vascular system, how it can be detected in vivo, and if it can be blocked by the common strategy for interrupting cellular interactions (delivering an inhibitor in solution) are questions yet outstanding.

The specific case of endothelial interactions with neutrophils illustrates that signaling may be required for cellular adhesion, that adhesive interactions between cells can spatially control and facilitate signaling, and that adhesion and signaling molecules work in sequence and in combination. Also, a balance between positive and negative signals may dictate the magnitude of adhesive interactions (5). These principles apply to interactions between other types of leukocytes and endothelial cells (a topic that will be revisited in a later perspective), to leukocyte–leukocyte interactions, and to interactions between structural cells of the vascular wall. Adhesive interactions between cells can also modify and integrate signals delivered through surface receptors, leading to functional outcomes that are different from those that occur when the target cell is stimulated in suspension. As an example, engagement of P-selectin glycoprotein ligand-1 (PSGL-1) on human monocytes by P-selectin on the surfaces of activated platelets causes the monocytes to respond to the chemokine RANTES by translocating NF-κB to the nucleus and synthesizing MCP-1 and other immediate-early gene products (17). There is little or no response to RANTES when monocytes are treated in suspension. This adhesion-dependent signal integration can be reproduced using purified P-selectin or transfected cells that express it and indicates that its counterligand, PSGL-1, is directly or indirectly linked to intracellular transduction systems in the monocyte that intersect with those linked to the serpentine receptor for RANTES. Adhesion-dependent signal integration has also been observed when β_1 or β_2 integrins on myeloid leukocytes bind to matrix proteins (3), appears to occur in angiogenic and apoptotic responses of endothelial cells when α_v integrins are engaged (18), and is a feature of other integrin-mediated interactions. However, the example involving P-selectin and PSGL-1 cited above, cellular interactions involving cadherins (19), and others make it clear that integration of signals from the external environment is not an exclusive province of integrins, although their identification heralded this property. The intracellular mechanisms involved in adhesion-dependent integration of extracellular signals likely include protein–protein interactions, scaffolding organizations of transduction cascades, and cytoskeletal alterations that are as varied as the intercellular interactions they control. The molecular basis of the interplay between adhesion and signaling, and how coordination between multiple adhesion molecules and signaling receptors that are simultaneously engaged is achieved (20), are key topics for the future.

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