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J Clin Invest. 1996;98(12):2661-2665. https://doi.org/10.1172/JCI119088.

Perspective

Perspectives Series: Cell Adhesion in Vascular Biology

Effects of Fluid Dynamic Forces on Vascular Cell Adhesion

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Cell adhesion plays a key role in a number of diverse biological processes, including inflammation and thrombosis. In the cardiovascular system, cells are exposed constantly to hemodynamic forces due to the flow of blood. Cell attachment to vessel wall depends on the balance between the dispersive hydrodynamic forces and the adhesive forces generated by the interaction of membrane-bound receptors and their ligands. Understanding the complex interplay among blood flow, cell adhesion, and vascular biology at the molecular level is crucial for developing specific approaches for altering vessel pathology. The extravasation of leukocytes from the vasculature to the tissue space is the pivotal event of the immune response and represents a relevant example of this dynamic adhesion process.

The multistep process of leukocyte extravasation: the neutrophil model

Upon activation the endothelium begins displaying specific adhesion molecules that bind free-flowing neutrophils (initial contact), slowing them down and causing them to roll in the direction of flow through labile contacts with the vessel wall (rolling). Initial contact and rolling of neutrophils along the endothelium are mediated predominantly by a class of adhesion molecules called selectins (Fig. 1). Selectins are transmembrane, carbohydrate-recognizing glycoproteins that constitute a family with three known members: E- and P-selectin expressed on activated endothelium, and L-selectin constitutively expressed on all leukocytes except for a subset of memory T cells. All three selectins bind in a calcium-dependent manner to sialylated and fucosylated oligosaccharides such as sialyl Lewis^x and its isomer sialyl Lewis^a. A human protein core ligand has been identified only for P-selectin. P-selectin glycoprotein ligand-1 (PSGL-1)[1] expressed on leukocytes belongs to the emerging family of adhesion molecules called sialomucins that are serine- and threonine-rich proteins heavily O-glycosylated. PSGL-1 and L-selectin ligands (in the murine system) require sulfation to mediate high-affinity binding to P- and L-selectin, respectively.

As neutrophils roll along the endothelium, they are exposed to activating signals, including soluble substances derived from the endothelium such as IL-8, agents presented on the endothelial cell surface such as platelet-activating factor, or direct signal transduction via adhesion receptors themselves. Neutrophil activation upregulates the binding affinity of a family of adhesion molecules called integrins via both conformational changes and altered interaction with the cytoskeleton. Activation-dependent attachment of integrin receptors on neutrophils to endothelial cell adhesion molecules of the immunoglobulin superfamily (intercellular adhesion molecule-1 [ICAM-1]) converts transient rolling interactions into firm adhesion (Fig. 1). Furthermore, upon activation neutrophils flatten on the endothelium, resulting in increased contact area for integrin-mediated binding and decreased fluid drag and torque on the cell. Subsequently, neutrophils undergo dramatic changes in shape allowing them to diapedese through the interendothelial junctions of the vessel wall (transmigration) (Fig. 1) and migrate to inflammatory sites. Each step of this multistage process is essential for the proper function of the immune system in humans.

The fact that different families of receptors are capable of mediating distinct types of adhesive events such as rolling or firm adhesion of leukocytes remained unknown until 1987, when Lawrence et al. (1) examined neutrophil adhesion to cytokine-stimulated endothelial cells under well-defined postcapillary venular flow conditions in vitro. These studies demonstrated that neutrophil primary adhesion (initial contact and rolling) to endothelium is mediated by β_2 integrin–independent mechanisms, whereas subsequent neutrophil firm adhesion/migration (secondary adhesion) is β_2 dependent (2). The initial flow studies were followed by many further studies, both in vivo using intravital microscopy and in vitro using flow chambers and videomicroscopy that indicate that (*a*) L-, E-, or P-selectins are capable of tethering free-flowing leukocytes; (*b*) L- and E- or P-selectins are required for optimal leukocyte rolling; and (*c*) β_2 integrins cannot initiate leukocyte adhesion under flow conditions, except possibly at wall shear stresses < 1.0 dyn/cm^2, but can support activation-dependent firm adhesion and subsequent migration.

Refinements of the four-step neutrophil model

Flowing neutrophils have been shown to tether and roll on the surface of other neutrophils bound to activated endothelium

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Received for publication 21 October 1996.

1. *Abbreviations used in this paper:* CR, consensus repeats; ICAM-1, intercellular adhesion molecule-1; PSGL-1, P-selectin glycoprotein ligand-1; VCAM-1, vascular cell adhesion molecule-1.

J. Clin. Invest.

0021-9738/96/12/2661/05 $2.00
Volume 98, Number 12, December 1996, 2661–2665

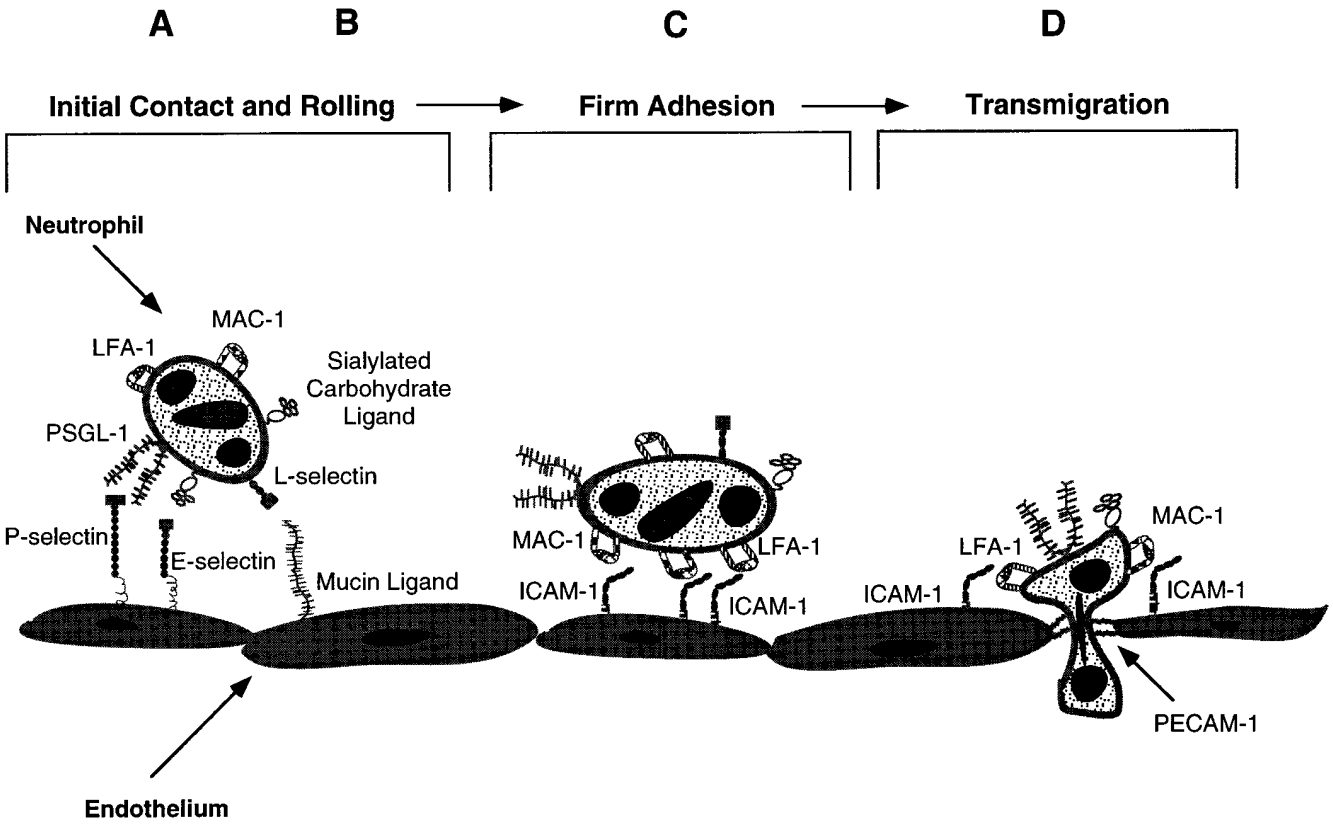

Figure 1. The four step model of neutrophil adhesion and transmigration across an endothelial monolayer under dynamic flow conditions at sites of inflammation. (*A*) Neutrophil tethering and (*B*) rolling (steps 1 and 2) are mediated by selectin–carbohydrate interactions. However, $\alpha_4\beta_1$ integrins, not expressed by resting neutrophils, are also capable of initiating primary lymphocyte adhesion to endothelial cells through binding to VCAM-1. (*C*) Firm adhesion (step 3) follows if neutrophils encounter activating signals while rolling along the endothelium. Activation-dependent attachment of β_2 integrins (Mac-1, LFA-1) on neutrophils to endothelial ICAM-1 supports this firm or secondary cell adhesion to the vessel wall. In addition, monocytes and lymphocytes may use the $\alpha_4\beta_1$/VCAM-1 pathway in this step. (*D*) Transmigration (step 4) does not necessarily accompany neutrophil adherence to the endothelium unless a favorable chemotactic gradient exists across the monolayer. Antibodies against β_2/ICAM-1 pathway that block firm adhesion exert a similar effect on neutrophil transendothelial migration. However, platelet/endothelial cell adhesion molecule 1 (PECAM-1) expressed at endothelial cell junctions appears to be required for transmigration by binding homophilically to PECAM-1 expressed on leukocytes and possibly heterophilically to an as yet unidentified receptor.

via L-selectin–dependent mechanisms (3). Walcheck et al. (4) extended these observations and demonstrated that free-flowing neutrophils can form homotypic tethers with neutrophils rolling on purified P-selectin before attaching to the substrate downstream of the previously rolling cells. These neutrophil–neutrophil interactions are mediated by L-selectin and can dramatically enhance the rate of cell accumulation on P-selectin, representing a potential additional pathway in the multistage process of neutrophil recruitment to sites of inflammation.

Several studies have been carried out to investigate whether lymphocyte- or monocyte-endothelial cell adhesive interactions under flow follow a multistep pattern analogous to that described for neutrophils. These studies indicate that mononuclear cells may use additional pathways for primary adhesion under flow. Lymphocytes and monocytes differ from neutrophils in that they express β_1 integrin receptors, including the very late antigen 4 ($\alpha_4\beta_1$). Jones et al. (5) demonstrated that $\alpha_4\beta_1$ integrins are capable of mediating primary T lymphocyte adhesion to vascular cell adhesion molecule-1 (VCAM-1) transfectants under flow conditions. This finding has been confirmed by others (6) who demonstrated that α_4 integrin transfectants are capable of tethering and rolling on VCAM-1 either immobilized on plastic or expressed on TNF-α–stimulated endothelium. Moreover, $\alpha_4\beta_7$ integrins can support lymphocyte attachment to mucosal addressin under shear forces in the absence of a selectin contribution (7). In contrast, the β_2/ICAM-1 pathway alone has been shown ineffective in initiating mononuclear cell–endothelial cell adhesive interactions at physiologic flow conditions (5–7), as shown earlier for neutrophils.

Simultaneous blockade of β_2 integrins and ICAM-1 significantly increases the velocity of neutrophils rolling either on histamine- or IL-1β–activated endothelial cells (8, 9). Recent in vivo studies have shown that leukocyte rolling is absent in P-selectin/ICAM-1 double knockout mice for a much longer time period than in P-selectin–deficient mice, suggesting that ICAM-1 may contribute to the leukocyte rolling contact with the vessel wall (10). Therefore, the steps of the molecular cascade of events seem to be overlapping and not strictly sequential.

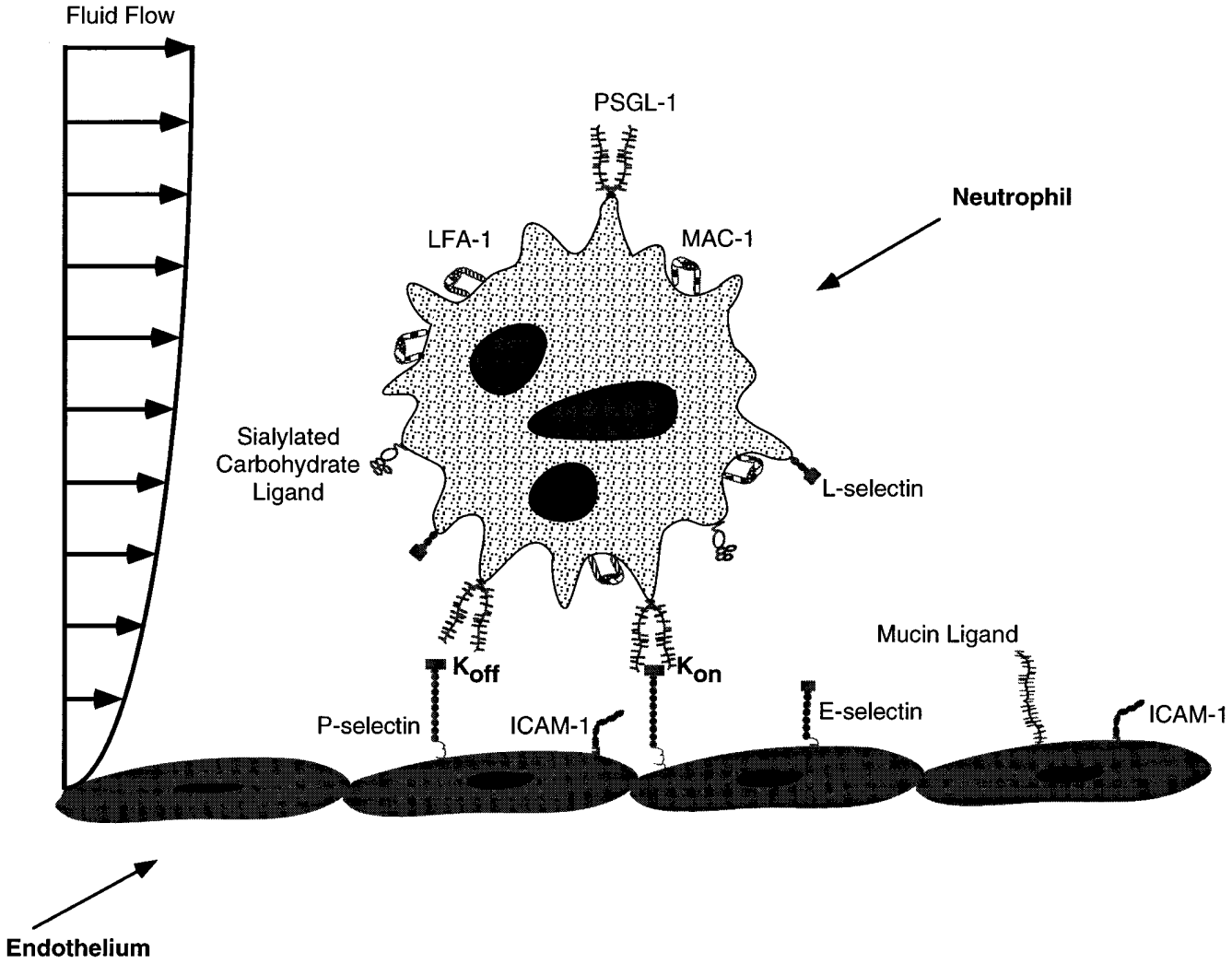

Figure 2. Neutrophil rolling on activated endothelium. Receptors mediating primary adhesion such as PSGL-1 and L-selectin are localized on the tips of microvilli on neutrophils, a position that is particularly advantageous for initial neutrophil-endothelial cell contact. In contrast, β_2 integrin receptors are displayed on the cell body and are insufficient to initiate adhesion under flow conditions. A rapid rate of bond formation (K_{on}) is necessary for neutrophil arrest from the bloodstream to the vessel wall. Furthermore, a fast dissociation rate (K_{off}) and the ability of the bond to withstand considerable strain before failing (K_{off} relatively insensitive to flow-induced stresses) will allow the captured neutrophil to advance forward in the direction of flow while remaining in contact (due to rapid K_{on} rates) with the vessel wall. Finally, the length of receptor/ligand bonds determines the ability of adhesion molecules to initiate tethering/rolling interactions under flow conditions.

Properties of the receptors mediating primary versus secondary adhesion

The leukocyte receptors involved in the multistep adhesion process are not randomly scattered on the cell surface, but are rather localized in or excluded from the tips of microvilli. L-selectin, PSGL-1, and α_4 integrins (at least the $\alpha_4\beta_7$) are concentrated on microvilli, whereas β_2 integrins are mainly displayed on the cell body of the leukocytes (Fig. 2). Receptor topography appears to regulate the efficiency of a molecule in initiating adhesion under conditions of shear. von Andrian et al. (11) demonstrated that although microvillous versus cell body epitope presentation does not play a role in cell binding under static conditions, it leads to a substantially differential cell capture under flow that is markedly evident at higher shear stresses ($\sim$ 3 dyn/cm^2). In addition, receptor clustering on microvilli of leukocytes may facilitate the involvement of these molecules in the earliest step of the adhesion cascade. It is thought that interaction of endothelial cells with leukocytes through microvillous receptors presumably reduces the area of initial contact and consequently the electrostatic repulsive forces generated by contacts between the glycocalyces of those cells (11). Furthermore, receptor clustering leads to the formation of multiple bonds between the interacting cells that may be required to provide the sufficient adhesive strength to resist shear forces that would lead to detachment.

As described by Bell (12), cell adhesion is mediated by reversible bonds between cell surface molecules and is dependent not only on the density, topographic localization of adhesion receptors, and the electrostatic repulsive forces between cells, but also on the kinetics of bond formation/dissociation, and the length and flexibility of the bonds. Selectins and α_4 integrins are capable of mediating the initial leukocyte tethering and rolling along the activated endothelium. To arrest leukocytes moving at high hydrodynamic velocity in the blood-

stream an extremely rapid rate of bond formation is necessary. Fast association and dissociation rates coupled with the ability of the bond to stretch before breaking under shear stress will allow the captured leukocyte to roll along the cell monolayer in the direction of flow (Fig. 2). The bond between P-selectin and its counterreceptor has fast association (1.5×10^7 s^{-1}) and dissociation (0.95 s^{-1}) rates (13). The modest increase of dissociation rate with shear stress, examined within the physiological range of 0.17–1.1 dyn/cm^2, demonstrates the high tensile strength of the selectin bond that is important for the maintenance of leukocyte rolling along the vessel wall.

It was demonstrated recently that projection of the ligand-binding domain of P-selectin well above the cell surface is required for optimal neutrophil capture under conditions of hydrodynamic flow (14). Using Chinese hamster ovary cells expressing P-selectin constructs in which various numbers of consensus repeats (CR) were removed, Patel et al. (14) showed that deletion of three or four CR minimally affected neutrophil adhesion, whereas deletion of five or more CR significantly impaired or abolished adhesion under flow, but not under static conditions. It is believed that the elongated molecular structure of P-selectin ($\sim$ 40 nm) facilitates contacts with its counterreceptor PSGL-1 ($\sim$ 60 nm long) on flowing neutrophils (Fig. 2).

Another important property of L-selectin that clearly differentiates it from β_2 integrins and makes this selectin specialized in initiating adhesion is that L-selectin can form functional bonds under flow without activation of leukocytes. This finding makes sense physiologically because circulating leukocytes are normally unactivated but can bind to specific endothelial sites when necessary. It is nonetheless possible that later activation can affect selectin adhesion. It has been shown, for instance, that formyl-Met-Leu-Phe stimulation of neutrophils induces a brief increase in L-selectin on the cell surface, followed by rapid proteolytic shedding. Furthermore, neutrophil activation by signaling molecules such as platelet-activating factor or IL-8 reduces adhesion to P-selectin. Decreased adhesion appears to be the result of redistribution of PSGL-1 on activated neutrophils mediated by cytoskeletal interactions and is not related to shedding of L-selectin (15). These processes may represent ways for the neutrophil to disengage selectin-mediated binding allowing free migration along the endothelial surface.

Several reports support the notion that selectins play a more complex role than merely catching leukocytes from flowing blood, and they have been implicated in signal transduction mechanisms. All three selectins have been shown to stimulate β_2 integrin adhesive responses. However, the detailed signaling mechanisms by which selectins interact with β_2 integrins remain to be elucidated.

Neutrophil adhesion to immobilized platelets is a sequential multistep process

The heterotypic interaction between neutrophils and platelets is presumably of pathophysiologic significance since it may promote thrombosis and vascular occlusion and prevent reflow in the microvasculature of ischemic regions. It is believed that platelets are induced to aggregate and to express P-selectin on their surfaces at sites of vascular injury, and the expressed P-selectin mediates neutrophil accumulation. It was shown recently that neutrophil attachment to activated platelet monolayers follows a multistep process of sequential involvement of distinct types of receptors analogous to the neutrophil–endothelial cell adhesion cascade (16). The initial adhesive interaction is mediated by P-selectin expressed on the surface of activated platelets and results in neutrophil rolling. However, neutrophils from healthy subjects but not leukocyte adhesion deficiency-I patients are arrested on immobilized platelets within 5–30 s after the initial attachment. Neutrophil activation is presumably induced upon platelet contact, but the underlying mechanism of this event remains to be elucidated. Once firmly adherent on activated platelets, neutrophils are able to migrate across the monolayer. Both firm adhesion and transplatelet migration have an absolute requirement for the β_2 integrin receptor Mac-1, but not for lymphocyte associated antigen-1 (LFA-1).

Platelet interactions with thrombogenic surfaces under flow: analogy to leukocyte adhesion

Platelet adhesion to exposed subendothelium at sites of vascular injury is a critical initiating step of the hemostasis and thrombosis. The specific platelet surface receptors involved in these processes are determined by the local hydrodynamic conditions and the extracellular matrix components. Savage et al. (17) demonstrated that platelet adhesion to immobilized von Willebrand factor (vWF) requires the sequential involvement of distinct receptor molecules analogous to the process of leukocyte extravasation in response to inflammatory stimuli. Platelet GPIbα seems to mediate a selectin-like initial adhesive platelet–vWF interaction, manifested as translocation along the wall. These activation-independent bonds presumably have fast association and dissociation rates and a high tensile strength, since they appear functional even at 6,000 s^{-1}. After platelet contact with the wall, $\alpha_{IIb}\beta_3$ becomes activated and binds to vWF leading thus to platelet permanent arrest onto the surface and subsequent thrombus formation. Furthermore, unactivated $\alpha_{IIb}\beta_3$ receptors are capable of supporting platelet attachment to immobilized fibrinogen only at low shear rates ($< 1{,}500$ s^{-1}), a fact that is attributed to a slow kinetics of bond formation and low tensile strength.

Concluding remarks

Despite the enormous progress made during the last decade in elucidating the underlying molecular mechanisms of leukocyte interactions with the vessel wall, our understanding of this process is far from complete. Cell adhesion molecules on both leukocytes and activated endothelium remain to be identified. These include the E-selectin ligand on leukocytes and the L-selectin inducible ligand expressed on IL-1β– or TNF-α–activated endothelium. It is of note that a ligand for E-selectin, E-selectin ligand-1, has been recently identified and cloned in the murine system. Furthermore, as suggested by Jones et al. (5, 8), a novel adhesion molecule yet to be determined, distinct from the L-selectin–inducible ligand, is expressed on 24-h IL-1β–activated endothelium and appears to play a key role in mediating T cell and neutrophil primary adhesion. Another interesting question raised in this work that deserves further investigation is the absence of leukocyte adhesion to 24-h IL-1β + IL-4–costimulated endothelial cells under flow conditions, in contrast to the substantial adhesion observed with either IL-1β– or IL-4–activated endothelium. One can speculate the synthesis of an antiadhesion molecule that abrogates leukocyte–endothelial cell interactions or the altered presentation of the endothelial receptors (i.e., steric hindrance due to differential glycosylation) to the free-flowing leukocytes.

At this point, our knowledge of the nanoscale biomechanics of vascular–endothelial cell adhesion is quite limited. Experimental work at the molecular level to determine the lifetime, interaction distance, and strain responses of the different families of adhesion receptor–ligand bonds is needed. Such information will provide direct evidence regarding the accuracy of our current models of leukocyte tethering/rolling and firm adhesion/migration. A better understanding of the molecular basis of vascular cell adhesion under flow conditions will provide insights for the development of new therapies for inflammatory and thrombotic disorders.

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