Platelet–Leukocyte Interactions in Cardiovascular Disease and Beyond

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Abstract—Platelet–leukocyte interactions define a basic cell process that is characterized by the exchange of signals between platelets and different types of leukocytes and that bridges 2 fundamental pathophysiological events: atherothrombosis and inflammatory immune reactions. When this process takes place at the site of atherosclerotic plaque development or at the site of endothelial injury, platelet-dependent leukocyte recruitment and activation contributes to the inflammatory reaction of the vessel wall, which accounts for the exacerbation of atherosclerosis and for intimal hyperplasia and plaque instability. Moreover, platelet–leukocyte interactions may have a key role in modulating a wide array of responses of both the innate and adaptive immune systems, thus contributing to the pathogenesis of inflammatory diseases and tissue damage, as well as to host defense. (Arterioscler Thromb Vasc Biol. 2010;30:2357-2361.)

Key Words: leukocytes ■ platelets ■ signal transduction ■ thrombosis ■ inflammation

Cross-talk between platelets and leukocytes is a common feature of atherothrombosis and inflammatory immune reactions. During the last 10 years, several authors have extensively reviewed the literature on platelet–leukocyte interactions, mainly focusing on their roles in cardiovascular disease.1,2 This present review initially briefly summarizes our recent advances in the understanding of the basic molecular mechanisms that underlie platelet–leukocyte communication. We then discuss selected studies that have defined the pathogenetic role of this process in animal models of vascular disease, inflammatory disorders of the respiratory tract, bowel and skin, glomerulonephritis, arthritis, and sepsis.

Routes of Platelet–Leukocyte Communication

Platelets communicate biochemical signals to neutrophils, monocytes, and subsets of lymphocytes through adhesive receptors and a multitude of secreted soluble mediators. Vice versa, leukocyte-released factors, including proteases and nitric oxide, can modulate platelet responses. On the one hand, platelet–leukocyte “transcellular metabolism” of arachidonic acid amplifies the synthesis of proinflammatory and vasoconstrictive compounds, such as the leukotrienes and thromboxane A2, and, on the other hand, it leads to the generation of lipoxins, which mediate the resolution of inflammation. Moreover, activated platelets may interact with vascular endothelium in several ways and induce expression of adhesive molecules and chemokines, which in turn mediate leukocyte recruitment.

The adhesive receptors that mediate the tight contacts between platelets and leukocytes have been characterized in detail. The initial contact is driven by the exposure of P-selectin on the activated platelets, which is recognized by P-selectin glycoprotein ligand (PSGL)-1 on the leukocyte surface. After ligation, PSGL-1 triggers activation-dependent conformational changes in the β2 integrins, which mainly involve Mac-1, and promote the firm adhesion of the neutrophils.3 In a similar way, platelet binding triggers the adhesiveness of β1 and β2 integrins in monocytes4 and promotes tethering of lymphocytes to peripheral lymph node addressin, thus facilitating lymphocyte delivery to high endothelial venules5 and lymphocyte homing during adaptive immune responses.6

The key molecular events that link PSGL-1 to β2-integrin activation were identified recently. The binding of P-selectin to PSGL-1 results in Src family kinase (SFK)-dependent phosphorylation of Naf-1 (Nef-associated factor 1), which is constitutively associated with the cytoplasmic domain of PSGL-1. The phosphorylated Naf-1 recruits phosphoinositide 3-OH kinase p85-p110δ, which then mediates Mac-1 activation.7 Moreover, a SFK-mediated, outside-in signal that is transduced by Mac-1 and that leads to phosphorylation of Pyk2 (proline-rich tyrosine kinase-2) is necessary to stabilize integrin adhesion.3,8

Leukocyte tethering by platelet P-selectin not only induces rapid β2 integrin activation but also triggers delayed responses, which include gene expression and protein synthesis; these are fundamental for leukocytes to acquire an inflammatory phenotype. Delayed responses require the concerted actions of outside-in signaling that is transmitted by adhesive receptors (mainly PSGL-1 and β2 integrins) and of...
signals transduced by chemokine or cytokine receptors. For example, P-selectin and RANTES act in concert to induce nuclear translocation of nuclear factor κB, gene expression, and synthesis of monocyte chemotactic protein (MCP)-1 and interleukin (IL)-8 in monocytes. More recently, Dixon et al demonstrated that prolonged interaction with activated platelets induces cyclooxygenase (COX)-2 expression in monocytes. In this model, binding of P-selectin to PSGL-1 triggers nuclear factor κB activation and transcription of COX-2 gene; a second signal elicited by IL-1β, which is also synthesized in the context of platelet-monocyte interaction, then mediates COX-2 mRNA stabilization and efficient COX-2 protein synthesis. Thus, platelet-induced signals finely regulate COX-2 expression in monocytes by acting at transcriptional and posttranscriptional checkpoints. Because COX-2–derived eicosanoids in monocytes may have deleterious effects in inflammation and atherothrombosis, this observation underscores a potential mechanism by which platelet–monocyte interaction may contribute to inflammatory syndromes and ischemic heart disease.

Platelets contain a multitude of chemokines that can be displayed on the cell surface or released as soluble molecules on activation, including the CC (RANTES, MCP-1, MIP-1α, TARC) and CXC (platelet factor-4, ENA-78, GROα) chemokines, β-thromboglobulin (converted to the CXC chemokine NAP-2 by neutrophil cathepsin G), CD40L, and TREM-1 (trIGGERING receptor expressed on myeloid cell-1) ligand (reviewed elsewhere). Within the close microenvironment between leukocytes and adherent platelet membranes, these mediators can activate their cognate receptors and induce immediate and/or delayed responses in immune cells (Figure 1).

In addition to direct cell–cell interactions, activated platelets can transfer released chemokines to the surface of inflamed or atherosclerotic endothelium. In this way, activated platelets leave a “message” on the vessel wall that can be “read” by circulating monocytes and can contribute to their recruitment and inflammatory activation at sites prone to atherosclerosis. Moreover, activated platelets disseminate microparticles, which are intact vesicles that form by budding from the membrane. As with whole platelets, these microparticles interact with leukocytes and other inflammatory cells and can amplify inflammation in human and experimental models of arthritis.

Collectively, the data discussed above indicate that platelets contribute to inflammation through leukocyte recruitment and activation. This is achieved by induction of integrin adhesion and chemotaxis; by stimulation of rapid responses, such as release of reactive oxygen species, myeloperoxidase, and proteases in neutrophils; and by inducing intracellular signals, leading to inflammatory and prothrombotic gene expression in monocytes. Through these common mechanisms, platelet–leukocyte interactions exacerbate vascular injury in atherothrombosis and tissue damage in a variety of inflammatory diseases.

**Platelet–Leukocyte Interactions in Atherothrombosis**

Circulating platelet–leukocyte aggregates are increased in acute coronary syndromes, and they may contribute to cardiac dysfunction after ischemia and reperfusion. Additionally, neutrophil infiltration at the culprit lesions in patients who have died following acute myocardial infarction suggests that platelet–neutrophil interactions occur at the site of ruptured plaques.

Recent studies in animal models have indicated a fundamental role for platelet–leukocyte interactions in the development and progression of atherosclerosis. In hypercholesterolemic animals, activated platelets and platelet–leukocyte aggregates adhere to the endothelium at sites that are prone to plaque formation and deliver RANTES and platelet factor-4; these in turn amplify the recruitment of monocytes and accelerate atherosclerosis. In vitro, activated platelets enhance the rate of cholesterol ester accumulation by cultured monocyte-derived macrophages, and they induce CD34+ myeloid progenitor cells to differentiate into foam cells.

Furthermore, platelet-mediated neutrophil recruitment promotes the development of intimal hyperplasia after experimental endovascular injury. Using a Dacron graft implanted within an arteriovenous shunt in baboons, Palabrica et al reported that P-selectin–mediated leukocyte accumulation in the developing thrombus promoted fibrin deposition. In vitro, platelet-derived CD40L and P-selectin provide signals that induce the expres-
sion of the procoagulant tissue factor in monocytes and granulocytes and stimulate procoagulant phosphatidylserine expression on the membrane of monocytes. In the mouse, high levels of soluble P-selectin stimulated the generation of leukocyte-derived procoagulant microparticles and induced a procoagulant state. Thus, platelet–leukocyte interaction may contribute to clot formation by mechanisms that are beyond the rapid formation of hemostatic plug.

**Platelet–Leukocyte Interactions in Inflammatory Lung Disease**

Platelet–leukocyte interactions occur in inflammatory lung disease. In a murine model of acute lung injury, platelet depletion or immunologic blockade of P-selectin reduced the histological changes and the protein leakage in the lung and reduced neutrophil accumulation in the intravascular, interstitial, and alveolar compartments, which indicated that platelet P-selectin mediates neutrophil recruitment. In vitro studies have suggested that thromboxane A2, which is released during platelet–neutrophil interactions, can stimulate intercellular adhesion molecule-1 expression by endothelial cells and the accumulation of neutrophils in the lungs. In a murine model of allergic inflammation, pharmacologically induced platelet depletion reduced eosinophil and lymphocyte accumulation in the lungs after exposure of animals to specific allergens. Here, leukocyte recruitment was completely or partially restored by injection of wild-type, and not P-selectin–deficient, platelets.

Clinical and experimental observations have suggested a role for platelet–neutrophil interactions in cystic fibrosis (CF), a genetic disease in which mutations in the gene of the CF transmembrane conductance regulator (CFTR) result in reduced secretion of Cl⁻ and HCO₃⁻ ions, thickened mucus secretion, and chronic infections of the airways. An excessive and persistent accumulation of neutrophils and tissue damage characterize the airways of patients with CF. These patients show increased levels of neutrophil–platelet and monocyte–platelet aggregates in their circulating blood and increased Mac-1 expression on their neutrophils and monocytes. Interestingly, in vitro blockade of CFTR in platelets and neutrophils from healthy subjects results in reduced generation of lipoxin A₄ by platelet–neutrophil coinulation and prolonged neutrophil survival, suggesting that dysfunctional platelet–neutrophil interaction may contribute to lung inflammation in CF.

**Platelet–Leukocyte Interactions in Inflammatory Bowel Disease**

Recent advances in animal models have highlighted a key role for platelet–leukocyte–endothelial cell interactions in the pathogenesis of inflammatory bowel diseases. Intravital video microscopy of colonic venules in mice subjected to experimental colitis has demonstrated that leukocytes that adhere to the vessel walls recruit the circulating platelets. The use of neutralizing monoclonal antibodies or the induction of colitis in P-selectin−/− mice indicated that P-selectin and PSGL-1 mediate the accumulation of platelets and leukocytes, and the extent of their accumulation correlates with disease severity. In addition to P-selectin–PSGL-1 pair, CD40L appears to mediate platelet–leukocyte interaction in experimental colitis. Genetic deletion or pharmacological inhibition of CD40-CD40L pathway reduces platelet and leukocyte accumulation in the colonic microvasculature and attenuate the disease. Thus, platelet and leukocyte recruitment in the microvasculature of the colon mucosa are codependent and may be responsible for microthrombosis, fibrin deposition, focal arteritis, and microinfarctions that have been observed in biopsies of patients with inflammatory bowel diseases. Hypercoagulability and the prothrombotic state of the inflamed mucosal microvasculature are also sustained by increased expression of the procoagulant tissue factor. In a mouse model of colitis, a blockade of tissue factor using monoclonal antibodies prevented platelet and leukocyte recruitment and reduced tissue injury and microthrombosis.

In patients with inflammatory bowel diseases, platelets circulate in an activated state, and they form heterotypic aggregates with circulating leukocytes and are an important source of inflammatory mediators. In addition to contributing to thrombotic and inflammatory reactions at the intravascular side of the intestinal microcirculation, platelets migrate across the mucosal epithelium together with neutrophils. Interestingly, transmigrated platelets release large amounts of ATP, which is metabolized to adenosine by ectonucleotidases expressed on the apical surface of intestinal epithelial cells. Adenosine, in turn, induces chloride secretion and concomitant water movement into the intestinal lumen. In this way, transmigrated platelets can affect important functions at the luminal surface of the epithelium.

**Platelet–Leukocyte Interactions in Inflammatory Skin Diseases**

Recently, the role of platelet–leukocyte interactions was investigated in a mouse model of chronic contact hypersensitivity. Flow cytometric analysis of the blood of the elicited animals showed increased P-selectin expression in circulating platelets and the formation of mixed platelet–leukocyte conjugates. Leukocyte recruitment and inflammatory skin reactions were significantly decreased in mice that were made thrombocytopenic, and they were restored by injection of platelets from wild-type, and not P-selectin–deficient, mice. Platelet-derived chemokines, MIP-1α, RANTES, and TARC, were also involved in leukocyte recruitment at the sites of skin inflammation. In a mouse model of cutaneous Arthus reaction, an inflammatory disease that is initiated by deposition of IgG-containing immune complexes, genetic deletion of P-selectin or PSGL-1 reduced leukocyte recruitment into the inflamed skin and reduced edema and hemorrhage in normal, but not in thrombocytopenic, mice, confirming that platelets cooperate in leukocyte recruitment into inflamed skin through the interaction of P-selectin with PSGL-1.

**Platelet–Leukocyte Interactions in Glomerulonephritis**

Detection of platelet-derived and neutrophil-derived cationic proteins in the glomeruli of patients with lupus nephritis and cryoglobulinemia-associated nephritis has suggested the involvement of both neutrophils and platelets in inflammatory glomerular diseases. In animal models of immune complex
extracellular traps to ensnare bacteria.38 (Figure 2).

host defense, through stimulating the formation of neutrophil platelets.

proinflammatory and prothrombotic potential of activated platelets by neutrophils,37 a process that plausibly limits the neutrophil adhesion triggers the phagocytic clearance of activated platelets,35 and they cooperate in the development of inflammatory reactions, which, when not controlled, can exacerbate tissue damage. Platelets may support lymphocyte homing in peripheral lymph nodes, stimulate isotype switching and production of IgG by B lymphocytes, and help lymphocyte responses to viruses and neutrophil response to bacteria. In this way, platelets contribute to host defense.

nephritis, which are characterized by diffuse proliferative nephritis and deposition of fibrin, neutrophils, and platelets colocalize into the glomerulus in a platelet P-selectin–dependent manner,35 and they cooperate in the development of renal injury.36

Concluding Remarks

This review supports the concept that platelet–leukocyte interactions have a pivotal role in promoting the inflammatory reactions of the vessel wall, which are fundamental for initiation and progression of atherothrombosis. Beyond atherosclerosis and acute thrombotic events, platelet–leukocyte interactions are involved in a range of inflammatory diseases, which supports the view that platelets are fundamental partners of the immune system. During these interactions, platelets trigger intracellular signaling in immune cells, and, in this way, they modulate inflammatory immune responses, most frequently by amplification. Moreover, platelet–neutrophil adhesion triggers the phagocytic clearance of activated platelets by neutrophils,37 a process that plausibly limits the proinflammatory and prothrombotic potential of activated platelets.

Finally, platelet–neutrophil interactions may contribute to host defense, through stimulating the formation of neutrophil extracellular traps to ensnare bacteria.38 (Figure 2).

New Pharmacological Avenues

Each of the molecular determinants of platelet–leukocyte cross-talk are promising pharmacological targets. Research on effective strategies targeting platelet–leukocyte interaction has been mainly focused on inhibitors of P-selectin. P-selectin antagonism showed efficacy in several animal models of disease, particularly in ischemia/reperfusion injury and arterial and venous thrombosis. As an example, monoclonal antibodies against P-selectin or a soluble recombinant form of PSGL-1, successfully paralleled enoxaparin for the treatment of deep vein thrombosis in nonhuman primates.39

The importance of platelet-derived chemokines in mediating platelet–monocyte communication highlighted the role of these chemokines as pharmacological targets to inhibit atherosclerosis. Koenen et al showed that selective disruption of RANTES–platelet factor-4 heterodimers by peptide inhibitors attenuates monocyte recruitment and reduces atherosclerosis in hyperlipidemic mice.40

Genetic and pharmacological targeting of the intracellular pathways that mediate PSGL-1–β2-integrin cross-talk have supported their pathophysiological importance in platelet-dependent granulocyte recruitment at the site of arterial injury8 and inflammation.7 This has, thus, emphasized the role of these molecular mechanisms, namely SFK- and phosphoinositide 3-kinase–mediated pathways that mediate PSGL-1–β2-integrin cross-talk: a molecular mechanism for polymorphonuclear leukocyte recruitment at the site of vascular damage.8

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Acknowledgments

We thank Christopher P. Berrie for editorial assistance, Matt Hazzard (Teaching and Academic Support Center, University of Kentucky) for preparation of the figures, and Roberta Le Donne for secretarial assistance. We also thank members of our laboratory for fundamental contributions to the work cited in this article.

Sources of Funding

This work was supported by the Fondazione Carichieti-Fondazione Negri Sud Onlus (to V.E.), the Ministero dell’Istruzione, dell’Università e della Ricerca, Decreto Ministeriale (MIUR D.M.) 44/08, Comitato Esperi Politiche della Ricerca, Decreto Direttoriale (CEPR D.D). 484/Ric 2008 (to V.E.), and National Institutes of Health grant HL080166 (to V.E.).

Disclosures

None.

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Arterioscler Thromb Vasc Biol. 2010;30:2357-2361; originally published online November 11, 2010;
doi: 10.1161/ATVBAHA.110.207480

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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