Platelet–Leukocyte Interactions in Inflammation and Thrombosis

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Abstract
Interactions between human platelets and leukocytes occur in physiological and pathological inflammation, hemostasis, and thrombosis. There is evidence that platelet–leukocyte interactions may be central mechanisms in atherosclerosis and its complications, and also in a variety of other acute and chronic diseases. In addition, models based on interactions of platelets and leukocytes are informative discovery tools that have yielded new insights relevant to human cell functions and cell-cell signaling and information transfer. This short article focuses on interactions between platelets and myeloid leukocytes and profiles some of the key pathways, mechanisms, and outcomes in these cellular dialogues.

Keywords
Platelets, leukocytes, monocytes, neutrophils, P-selectin, integrins, signaling, inflammation, hemostasis, thrombosis, atherosclerosis

Observations Using Human Platelets, Monocytes, and Polymorphonuclear Leukocytes
Platelets store, synthesize, and release a number of factors involved in inflammatory events. In the most well-known mechanism, platelets translocate factors stored in subcellular granules to the plasma membrane or release them into the circulation. Factors released into solution can activate leukocytes and other ‘target’ cells in a paracrine fashion. In addition, molecules retained on the platelet surface can mediate direct intercellular signaling without being released: so-called juxtacrine signaling. Once expressed on the membrane, some factors are shed by cleavage and/or are released into the milieu associated with platelet-derived microparticles, and are then able to alter functions of target cells.

In 1986, Jungi et al. reported that thrombin stimulation of platelets induces translocation and expression of surface proteins, which interact with ligands (‘counter-receptors’) on monocytes and polymorphonuclear (PMN) leukocytes (neutrophils), resulting in intercellular adhesion. The binding was influenced by temperature and was dependent on extracellular Ca²⁺. Many subsequent reports have confirmed this observation and mechanisms that are involved have been identified. P-selectin (CD62P, previously known as GMP-140 and PADGEM), a member of the selectin family of adhesion molecules, is of central importance in adhesive interactions between platelets and leukocytes. P-selectin is
stored in α-granules in quiescent circulating platelets and is translocated to the plasma membrane upon activation. P-selectin glycoprotein ligand-1 (PSGL-1) is a specific ligand for α- and β-selectins, and is expressed on monocytes and PMNs. Binding of P-selectin to PSGL-1 mediates platelet adhesion to neutrophils and monocytes. Proteinase-activated receptor (PAR) agonists such as thrombin, thrombin-receptor-inhibiting peptide (TRAP), PAR1AP, and PAR4AP induce the formation of platelet–leukocyte aggregates by stimulating P-selectin exposure on the surface of activated platelets and microparticles. P-selectin–mediated adhesion of platelets to myeloid leukocytes triggered in this fashion is responsible for stable and strong interactions. Other agonists also induce P-selectin display on activated platelets with variable potency compared with PAR agonists. Monocytes bind α-granule-expressing platelets more rapidly than PMNs, and platelet–monocyte aggregates form even when weaker platelet agonists are used. Activated platelets bind not only monocytes and neutrophils but also eosinophils, basophils, and subpopulations of T cells. Like platelet–monocyte and platelet–PMN aggregates, such aggregates are detectable by flow cytometry. P-selectin on the surface of activated platelets also mediates these adhesive interactions.

In addition to forming heterotypic aggregates in solution, leukocytes adhere to immobilized, activated platelets under shear stress conditions intended to model blood flow. Adhesion of PMNs under these conditions is reduced using antibodies against P-selectin or ethylenediaminetetraacetic acid (EDTA) to chelate extracellular Ca²⁺, which is required for binding of P-selectin to PSGL-1. Platelet-activating factor (PAF) translocated to the surface of collagen-adenosine 5′-monophosphate (cAMP)-mediated neutrophil activation and firm adhesion to platelet monolayers. Additional adhesive and signaling mechanisms are also involved.

In addition to P-selectin/PSGL-1, other molecular interactions mediate intercellular interactions between activated platelets and myeloid leukocytes (see Figure 1). For example, soluble agonists and P-selectin–PSGL-1 binding can alter the activation state of a β₃ integrin, αMβ2 (CD11b/CD18, Mac1), on human myeloid leukocytes. Fibrinogen can then be bound by activated αMβ2 on PMNs and monocytes and also by integrin αMβ2 (GPIIb/IIIa) on the activated platelets, thereby mediating adhesion of the two cell types. As a second example, GPIbα and junctional adhesion molecule 3 (JAM-3) on the platelet plasma membrane bind directly to αMβ2 displayed by myeloid leukocytes. Recently, it was reported that microtubules bearing activated αMβ2 are released by PMNs and can activate resting platelets by engaging GPIbα; this adhesion and signaling event acted in concert with P-selectin–PSGL-1 binding. Thus, platelet–leukocyte interactions can be bi-directional.

Platelets constitutively express intercellular adhesion molecule-2 (ICAM-2) but not ICAM-1 or -3 on their surfaces. Although the level of expression of ICAM-2 on the plasma membrane of platelets does not change with activation, it serves as a counter-receptor for another member of the leukocyte β₂ integrin family, αMβ2 (leukocyte function-associated antigen-1 [LFA-1]), which can mediate activation of leukocyte adhesive aggregates to platelets. Thrombomodulin (TSP) is a platelet α-granule protein that, similar to P-selectin, is translocated to the plasma membrane upon activation; it is also an antibody against TSP-inhibited platelet–monocyte aggregate formation. Thus, additional molecular pathways besides P-selectin–PSGL-1, integrins, ICAMs, and platelet glycoproteins mediate membrane contact and interactions between platelets and leukocytes. As noted above, many factors such as chemokine (C-C) ligand 5 (CCL5, also known as RANTES), soluble CD40 ligand (sCD40L), matrix metalloproteinases (MMPs), platelet factor 4 (PF4), and interleukin 1 receptor antagonist proteinase-activated receptor (IL-1Ra) that are released into the milieu by activated platelets and can provide signals to leukocytes without direct cell-cell interaction. Nevertheless, these paracrine factors and/or soluble factors released by leukocytes themselves or other cell types can act in concert with signals delivered by P-selectin–PSGL-1, integrins, or other adhesion ligands, providing a mechanism for signal integration and amplification.

Platelet–Leukocyte Interactions—Animal Models

In vivo animal models have been used to study platelet–leukocyte interactions in inflammatory and thrombotic events. Apolipoprotein E-deficient (ApoE⁻/⁻) hypercholesterolemic mice are commonly employed to examine development of atherosclerosis. Recently, it was reported that these animals are characterized by early adhesion of platelets to the endothelium, resulting in recruitment of leukocytes to the carotid artery, suggesting that this is critical in the initiation of atherosclerotic disease.
Thrombosis

Table 1: Clinical Conditions in Which Platelet–Leukocyte Interactions Have Been Reported or Platelet–Leukocyte Aggregates Have Been Detected in the Circulation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Aggregates Detected</th>
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<tbody>
<tr>
<td>Atherosclerosis</td>
<td>Sepsis</td>
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<tr>
<td>Acute coronary syndromes</td>
<td>Acute lung injury</td>
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<tr>
<td>Percutaneous coronary interventions</td>
<td>Cystic fibrosis</td>
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<tr>
<td>Cerebrovascular ischemia and stroke</td>
<td>Complications of transfusion</td>
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<tr>
<td>Venous stasis ulceration</td>
<td>Hip arthropathy</td>
</tr>
<tr>
<td>Smoking</td>
<td>Ulcerative colitis</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Cirrhosis</td>
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lesion development. Furthermore, animal studies indicated that platelet–leukocyte aggregates form and associate with endothelium in response to cigarette smoke, a known environmental risk factor for atherosclerosis.

In models of vessel injury, P-selectin-deficient mice accumulate fewer leukocytes, resulting in protection against intimal hyperplasia and reduced number and size of atherosclerotic lesions. ApoE-/- mice transplanted with bone marrow from P-selectin-/- mice had 30% smaller atherosclerotic lesions than ApoE-/- mice transplanted with P-selectin+/+; marrow, this was interpreted as indicating a role for platelet-expressed P-selectin in atherosclerosis lesions. Consistent with this possibility, one potential explanation is adhesion of activated platelets to myeloid leukocytes and recruitment of these cells to the evolving atherosclerotic plaques. In a different study, infusion of activated platelets into C57BL/6 mice increased the number of circulating leukocyte–platelet aggregates. This induced monocyte and, to a lesser extent, neutrophil accumulation in the atherosclerotic lesion, indicating that circulating activated platelets and platelet–leukocyte aggregates promote the evolution of atherosclerotic lesions. Intercellular signaling between platelets and myeloid leukocytes in vivo may be required for their local accumulation in experimental vascular injury. Such adhesion and signaling interactions occur in both experimental and clinical angioplasty and vascular stent placement. Animal models also suggest important roles for platelet interactions with leukocytes in other inflammatory syndromes besides atherosclerosis and its sequelae. For example, several studies indicate that this is a feature of murine models of acute lung injury and experimental acute respiratory distress syndrome. Nevertheless, it should be remembered that there are differences in circulating platelet and leukocyte numbers and perhaps function in mice and other species, which may influence the clinical relevance of outcomes in these experimental systems.

Functional Consequences of Platelet–Leukocyte Interactions in Inflammation and Thrombosis—Intercellular Signaling and Signal Transduction

Platelet–leukocyte interactions appear to be mechanisms for targeting and local accumulation of leukocytes in physiological inflammation and hemostasis, and for local or systemic cell–cell contact in pathological inflammatory and thrombotic syndromes. It has also been suggested that formation of platelet–leukocyte aggregates contributes to the clearance of activated cells from the circulating blood. In pathological conditions in which platelet–monocyte or platelet–neutrophil aggregates form and are retained in the vasculature, these multicell clusters may be involved in mechanical occlusion of vessels, such as coronary arteries or lung capillaries. However, in addition to mechanical consequences, platelet–leukocyte interactions can alter the functional responses of both the leukocyte and the platelet. For example, platelet activation resulting from signals delivered by myeloid leukocytes or leukocyte microparticles may amplify thrombosis by triggering αIIbβ3-dependent adhesive events and surface expression of P-selectin. P-selectin is reported to act synergistically with tissue factor, which is generated in platelet–monocyte interactions and by activated platelets, thereby accelerating fibrin deposition. Conversely, it is clear that signals delivered to monocytes or PMNs by activated platelets can induce functional responses by the leukocytes that have inflammatory and thrombotic consequences. Some of these signaling mechanisms have been characterized relatively recently, while others are still in the process of being identified and dissected.

Signals from activated platelets induce expression of nuclear factor kappa B (NF-κB)-dependent genes by human monocytes. An early report described synthesis of monocyte chemotactic protein 1 (MCP-1) by human monocytes adherent to thrombin-stimulated platelets in heterotypic clusters. The mechanism involves parallel signaling via P-selectin–PSGL-1 engagement and the soluble chemokine RANTES, which is released from activated platelets and is recognized by CC chemokine receptor 5 (CCR5) on the monocyte (see Figure 1); these signals are integrated to yield transcription of the messenger RNA (mRNA) for MCP-1 and synthesis of the protein. Monocytes also synthesized IL-8 and tumor necrosis factor alpha (TNF-α) under these conditions.

More recently, engagement of PSGL-1 on human monocytes by P-selectin and parallel signaling by IL-1β were shown to mediate biphasic and temporally regulated expression of cyclo-oxygenase 2 (cox-2) in adhesive interactions of the leukocytes with thrombin-stimulated platelets. Apart from thrombin, additional agonists trigger formation of human platelet–monocyte aggregates and consequent chemokine synthesis. Early and more recent studies using primary monocytes and myeloid cells transfected with reporter constructs demonstrated that engagement of PSGL-1 by P-selectin signals to specialized translational control checkpoints. For example, adhesion of human monocytes to purified, immobilized P-selectin or to activated platelets in heterotypic clusters may amplify thrombosis by triggering αIIbβ3-dependent adhesive events and surface expression of P-selectin. P-selectin is reported to act synergistically with tissue factor, which is generated in platelet–monocyte interactions and by activated platelets, thereby accelerating fibrin deposition. Conversely, it is clear that signals delivered to monocytes or PMNs by activated platelets can induce functional responses by the leukocytes that have inflammatory and thrombotic consequences. Some of these signaling mechanisms have been characterized relatively recently, while others are still in the process of being identified and dissected.

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transcriptional and post-transcriptional mechanisms in a gene-specific and temporally controlled fashion. Specific pathways may thus be targets for therapeutic intervention in pathological inflammation involving platelet–monocyte signaling. Similar functional responses also occur in models in which PMNs interact with activated platelets, although cell-specific variations undoubtedly occur. For example, earlier and more recent observations indicated that engagement of PSGL-1 on human PMNs activates mitogen-activated protein (MAP) kinases, and that PSGL-1 in parallel with the PAF receptor can signal to components of the mTOR pathway in human neutrophils. Thus, intracellular transduction cascades in PMNs are activated by signals from platelets. Taken together, the studies also suggest that platelet–PMN interactions may induce synthesis of chemokines and other inflammatory factors by PMNs when platelet–neutrophil aggregates form or PMNs adhere to platelets at sites of vascular injury. The findings further suggest that in PMNs, as with monocytes, both transcriptional and post-transcriptional pathways are triggered by signals from activated platelets. Conversely, it is unknown whether signaling by adherent PMNs or monocytes induces post-transcriptional expression of new proteins by the interacting platelets, mediated by transduction pathways that have recently been described. Nevertheless, the evidence for altered gene expression and synthesis of inflammatory and hemostatic proteins in platelet–leukocyte interactions that we have briefly reviewed here adds to the complexity of synthetic responses, including production of biologically active lipid mediators and other factors, that have been previously recognized in molecular dialogues between these cell types.

Platelet signaling of leukocytes may induce additional intracellular responses relevant to inflammation and thrombosis. Several reports indicate that interactions of monocytes with platelets in vitro alter cell fate determination as the leukocytes are induced to differentiate toward macrophages or dendritic cell lineage endpoints. Thus, platelet–leukocyte interactions have the potential to broadly influence inflammatory responses by altering the developmental pathways of specialized leukocytes that are major regulators of innate and acquired immune functions and that mediate interplay between coagulation and inflammation.