Platelets: Lone Rangers of Inflammatory Signaling in the Lung

Beyond their traditional regulatory role in thrombosis and hemostasis, platelets are now also recognized to be effectors in immunity and inflammation (1, 2). Reports of dysregulated inflammation and coagulation, key pathobiological features of acute respiratory distress syndrome (1, 3), have led to an effort to elucidate the role of platelets in propagating lung injury and to establish platelets as targets for therapeutic manipulation. Platelets participate in the immune response via signaling through many of the receptors and ligands that "traditionally" mediate hemostasis (2). For example, GPIIb-IIIa, which enables platelet adhesion and fibrin clot stabilization among other procoagulant activities, also mediates degranulation and the release of various proinflammatory factors, including IL-1b (2). Likewise, signaling through P2Y, TXA2, and PAF receptors mediates platelet aggregation but also facilitates the release of IL-1β and chemokines, and platelet–cell interactions with the endothelium and leukocytes within the immune cascade (4–6).

The directionality of platelet–leukocyte interactions appears to vary with the experimental context. Platelet recruitment to the lung was shown to be neutrophil dependent in transfusion-related acute lung injury and some models of peritonitis-induced lung injury (7, 8), whereas lung accumulation of neutrophils was shown to be platelet dependent in models of lung injury due to sepsis and sickle-cell disease (9, 10). In this issue of the *Journal*, Cleary and colleagues (pp. 232–243) report on a series of experiments to address whether lung platelet recruitment requires neutrophils in a murine model of lung injury induced by inhaled LPS (11). After LPS inhalation, platelet and neutrophil staining was increased in the lung, with a significant increase in the fraction of platelets that are associated with neutrophils (Figure 1A), although the majority (>70%) of lung platelets appeared to be unassociated with neutrophils. Disruption of the platelet–neutrophil interaction was achieved through blockade of p-selectin/PGSL-1 signaling (Figure 1B), the prominent pathway for platelet–leukocyte binding, or depletion of neutrophils with anti-Ly6G antibody (Figure 1C). With antibody blockade of p-selectin, PGSL-1, or neutrophil depletion, lung neutrophil recruitment after LPS was diminished, whereas lung platelet recruitment remained unchanged (Figures 1B–1C), suggesting that platelet recruitment was not dependent on neutrophils. Using intravital microscopy to visualize and quantify cell recruitment in mice with genetically labeled platelet membranes (Pf4-Cre x mTmG), the authors directly showed increased platelet migration and adhesion events in the live lung after LPS. Importantly, platelets that migrated into the lung after inhaled LPS were not aggregated and appeared visually distinct from platelet adhesion events induced by thrombosis (thrombin injection). Thus, these results not only confirm the expansion of lung platelets in LPS-induced lung injury, as revealed by an innovative combination of imaging and transgenic mouse techniques, but also extend the evidence for platelets' participation in the inflammatory cascade independently of other dominant effector cells.

In addition to neutrophil independence, platelet accumulation in the mouse lung after inhaled LPS is shown by Cleary and colleagues to be independent of thrombin activation. As such, the exact mechanism underlying recruitment and adhesion of platelets to the inflamed lung remains to be elucidated and represents an avenue of further study. Indeed, platelets themselves express Toll-like receptors and are capable of responding independently to pathogenic triggers, such as LPS (1, 2, 12). Furthermore, the adhesion of platelets to endothelial cells does not require vessel damage, as in the case of hemostasis, but can result from signaling between activated platelets and endothelium that promotes the surface expression of adhesion molecules (13). Thus, platelets possess the molecular machinery to independently respond to pathogenic signals and attach to inflamed endothelium as they traverse the lung vasculature. Lastly, the lungs are now established as a site for platelet biogenesis, with recent work using intravital microscopy providing direct visualization and quantification of intrapulmonary platelet release from megakaryocytes that originate from bone marrow but reside in the lung after LPS-induced lung injury (14). Cleary and colleagues provide supplemental data showing intrapulmonary megakaryocyte thrombopoiesis events that may represent an important source of platelet accumulation in lung inflammation. Although it is still relatively unexplored within the lung, inflammatory signaling through Toll-like receptors expressed on megakaryocytes and via cytokines, such as IL-1β, can influence megakaryocyte maturation and thrombopoiesis (15).

Acute respiratory distress syndrome is a lung disease of high morbidity and mortality for which there is currently no preventive or curative targeted treatment. Preclinical studies, such as that conducted by Cleary and colleagues, solidify the platelet's role in the inflammatory cascade and reveal aspects of platelet signaling as targets for pharmacological manipulation. Treatment with aspirin was shown to attenuate inflammation in experimental transfusion-related, LPS-induced, and ischemia/reperfusion lung injury (2). An additional translational application of platelet biology capitalizes on their intimate association with the site of injury as well as their accessibility via the circulation, allowing the use of platelet function as a biomarker that tracks with disease activity. Beyond the pulmonary alveolar space, platelets contribute to disease pathogenesis in the airway and pulmonary vascular compartments. For example, in airway inflammation, platelets influence bronchoconstriction and airway remodeling in asthma, and increased circulating platelet-leukocyte aggregates and indices of activation can be detected during asthma flares (9, 16). Indeed, the use of platelets as a marker, as well as to study human lung disease, is gaining popularity.

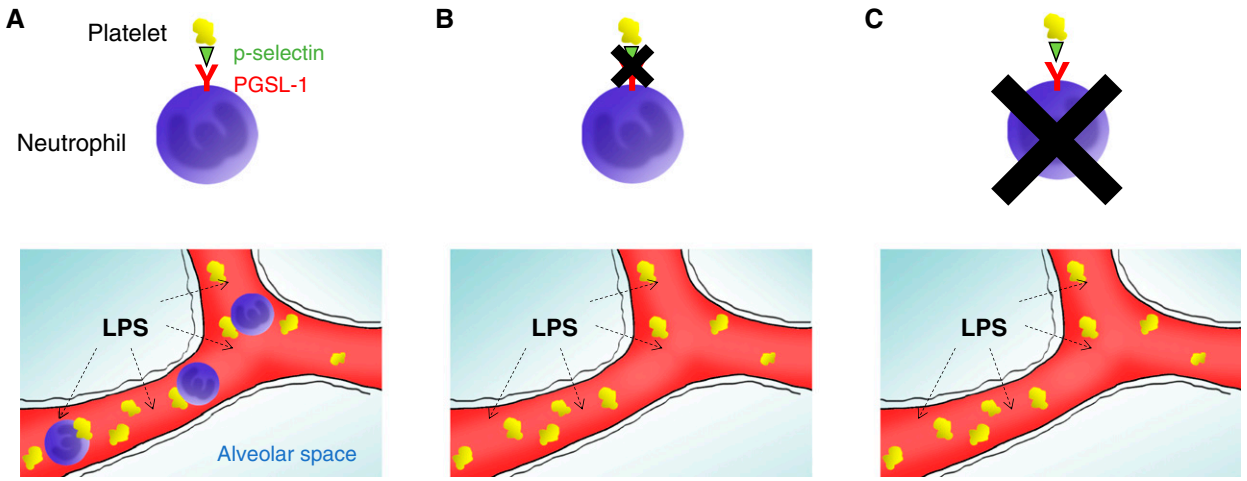

Figure 1. Inflammatory platelet recruitment to the lung is neutrophil independent. (*A*) After inhalation of LPS, platelets and neutrophils are both recruited to the lung. (*B* and *C*) Platelets accumulate in the lung even when binding with neutrophils is disrupted (*B*) or neutrophils are depleted (*C*). PGSL-1 = p-selectin glycoprotein ligand-1.

In conclusion, the platelet is gaining a firm position within the inflammatory cascade, where its repertoire of signaling through receptors, surface adhesion molecules, and granule release allows for responses that are both independent of and orchestrated with other inflammatory cell types. Adding to our mechanistic knowledge of platelet responses in lung injury, Cleary and colleagues lay the groundwork for novel translational applications of platelet biology in monitoring and treating lung disease. ■

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