Amicus or Adversary

Platelets in Lung Biology, Acute Injury, and Inflammation

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Platelets are the chief effector cells in hemostasis and have additional major functions in inflammation, vascular integrity, and tissue repair. Platelets and the lungs have interrelated activities. Previous studies provide evidence that platelets contribute to pulmonary vascular barrier function and are required for defense against pulmonary hemorrhage, and that the lungs can influence platelet number and distribution. There is also evidence that platelets contribute to pathologic syndromes of pulmonary inflammation and thrombosis. Thus, platelets have an "amicus or adversary" relationship with the lung. Recent observations and discoveries have established new paradigms relevant to influences of platelets on lung cell and molecular biology. These new findings are in a variety of areas including thrombopoieis, nontraditional activities of platelets, new synthetic capabilities and mechanisms of post-translational gene expression, interactions of platelets with endothelial cells and contributions to alveolar capillary barrier permeability, interactions of platelets with myeloid leukocytes, and platelet involvement in stem cell signaling and vascular repair. These issues are considered in a translational approach, with an emphasis on acute lung injury and the acute respiratory distress syndrome.

Keywords: platelets; inflammation; thrombosis; lung injury

Platelets are anucleate cells of myeloid origin that circulate in a resting state in the blood (1). In response to activating signals, the quiescent platelet phenotype changes and these versatile effectors of host defense perform major hemostatic, inflammatory, and reparative functions (2, 3). Platelets and the lung—a complex multicellular organ that accomplishes gas exchange, immune surveillance, and other physiologic processes—have interrelated activities (Figure 1). Platelets and platelet precursors transit the normal and injured mammalian lung. Inflammatory diseases and other pulmonary disorders cause accumulation of platelets in the lung, and remote tissue injury and systemic conditions such as sepsis can activate platelets in the circulation and cause them to sequester in pulmonary vascular beds. Activated platelets release paracrine mediators that can alter pulmonary function in a variety of ways. In addition, adhesion molecules and other factors with signaling properties are locally displayed on surfaces of activated platelets, where they can bind to ligands on target cells and trigger functional responses in a spatially localized, juxtacrine fashion (2, 4). These adhesion and signaling molecules allow platelets to interact not only with pulmonary endothelium and, potentially, other resident lung cells, but also with one another and with leukocytes in pulmonary vessels.

CLINICAL RELEVANCE

The studies reviewed here will alter paradigms regarding the contributions and activites of platelets in acute lung injury and other syndromes of pulmonary inflammation and thrombosis.

A variety of platelet–lung interactions were identified in early studies (5) and summarized and cataloged over a decade ago (reviewed in Ref. 6). More recently, new discoveries and insights into previously unrecognized features of platelet function have emerged. These observations alter concepts and paradigms regarding contributions of platelets to respiratory cell and molecular biology and to pulmonary diseases. This translational review will consider some of these recent findings, concentrating on those that are particularly relevant to the clinical spectrum of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) (7). Because of space limitations we refer to published work in a focused and eclectic, rather than comprehensive, fashion. Roles of platelets in ALI/ARDS have been reviewed previously (6, 8, 9) and additional relevant reviews are cited when possible in our discussion of specific topics.

PLATELET ONTOGENY AND THROMBOPOIESIS: MARROW, BLOOD, AND LUNG

Hematopoietic stem cells differentiate to immature megakaryocytes, which are lineage-committed parent cells that then mature and spawn platelets in the process of thrombopoiesis (1, 10). Platelet production can increase by as much as 20-fold in conditions of peripheral demand and inflammation (11). Discovery of the cytokine thrombopoietin, and identification of transcription factors that influence megakaryocyte differentiation and the expression of platelet-specific proteins, were critical in understanding the regulation of thrombopoiesis (10, 12). Two theories of terminal platelet formation have been proposed based on studies conducted in model systems and *in vivo* over many years (11). In one, cytoplasmic fragmentation of megakaryocytes in the bone marrow and pulmonary capillary bed is thought to generate mature, circulating platelets (13, 14). A potential problem with this idea is that the intracellular composition of mature platelets might then be predicted to be

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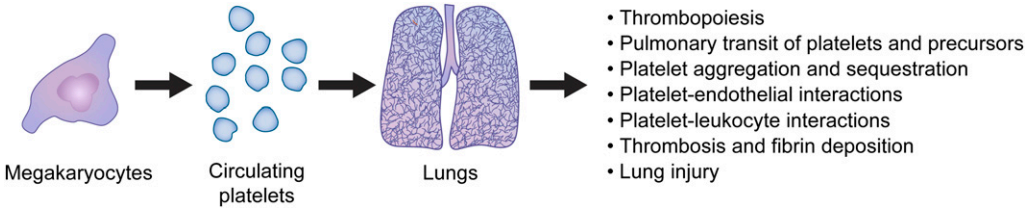

Figure 1. Platelets and the lungs interact in physiologic and pathologic conditions. Platelets, platelet precursors, and the lungs have activities that lead to multiple levels of interaction under physiologic condition and in acute lung injury (ALI)/acute respiratory distress syndrome (ARDS). Each of the processes and interactions listed is considered in the text.

random unless megakaryocyte cytoplasmic composition is uniform and fragmentation is a stereotyped process. A second model proposes that differentiated marrow megakaryocytes extend multiple elongated processes termed proplatelets; platelets form at the ends of proplatelets after regulated translocation of intracellular constituents to proplatelet tips via microtubular tracks (reviewed in Refs. 1 and 10) (Figure 2). Studies of differentiation of mouse and human hematopoietic stem cells to megakaryocytes *in vitro* support this second model (1, 10, 15–18). The two models of thrombopoieis are not necessarily mutually exclusive (13).

A recent study using intravital microcopy to examine exposed bone marrow of thrombopoietin-treated transgenic mice reported proplatelet formation *in vivo* (19), confirming previous evidence for this mechanism (1, 10, 13). Sessile megakaryocytes labeled with an expressed florescent protein were observed to extend proplatelets into marrow sinusoidal blood vessels, where shear forces appeared to free them from the parent cells. Circulating cells with proplatelet morphology were present in the systemic blood of these animals, suggesting the possibility that generation of single, mature platelets continues in peripheral vessels. Additional findings supported observations dating to the first half of the 20th century indicating that platelet counts are higher in pulmonary venous samples compared with pulmonary arterial blood, suggesting that circulating proplatelets undergo processing to two or more mature platelets in the lungs (5, 11, 19, and references cited therein). This study did not directly address the molecular basis for the fact that normal wild-type mice have circulating platelet counts 4- to 5-fold greater than those in humans (20).

How thrombopoiesis is altered in inflammatory lung diseases, and what the consequences are, remain largely unknown. Circulating platelet numbers may influence the natural history of a variety of pulmonary syndromes, including cystic fibrosis (21), asthma (22), pulmonary hypertension (23), and ALI/ARDS. Increased platelet turnover rates, altered platelet life span, thrombocytopenia, and thrombocytosis have each been reported in patients with ALI and/or ARDS (14, 24–26). Whether or not proplatelets circulate and give rise to individual platelets in an altered fashion in the blood in ALI/ARDS is unknown. Furthermore, there are additional mechanisms of platelet generation in the peripheral circulation (H. Schwertz and coworkers, unpublished data) that may be influenced by inflammatory lung injury.

There is evidence that the lung is a reservoir for megakaryocytes (10, 13) in addition to being a site of proplatelet processing. Megakaryocytes are reported to circulate and accumulate in the lungs of humans and rodents (27). A pathologist with a busy lung biopsy practice might note pulmonary intravascular megakaryocytes in several specimens a day across the spectrum of normal and diseased lungs, and would also identify them in autopsy examinations (T. Colby, M.D., personal communication). A recent retrospective analysis of thorascopic biopsy specimens from patients in the fibroproliferative phase of ARDS indicated that megakaryocytes are present in microvessels of the injured lung, and suggested that intrapulmonary megakaryocytes may influence circulating platelet numbers (14). Patients with thrombocytopenia had greater mortality than those with thrombocytosis in this series. Generalization of these observations is complicated by the fact that few of these patients had common causes of ALI/ARDS such as sepsis, trauma, or aspiration (7), and drug-induced diffuse alveolar damage was the etiology in approximately a quarter of the small group of subjects (14). Furthermore, the histochemical marker used, GP IIIa (CD61; β_3), is not specific for megakaryocytes and platelets, although it is present on these cells complexed to GPIIb (α_{IIb}). Nevertheless, these observations suggest that megakaryocytes are present in the lungs of patients in the

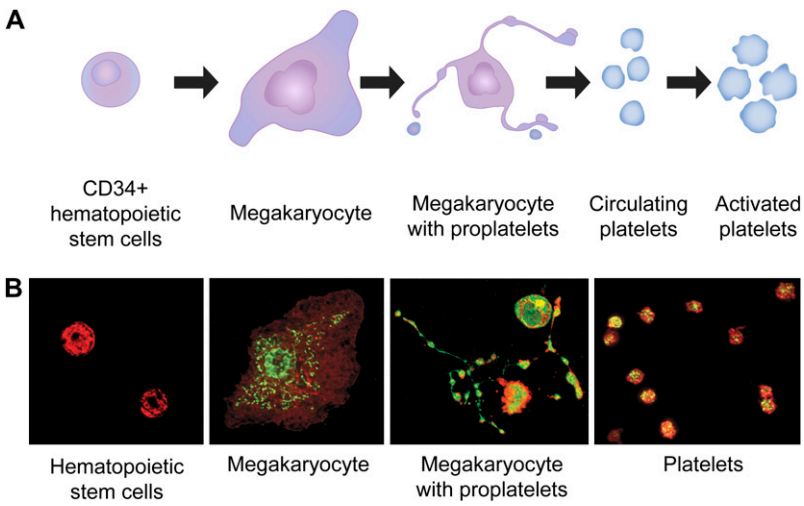

Figure 2. Thrombopoiesis involves regulated proplatelet formation and release of mature platelets. (*A*) $CD34^+$ hematopoietic stem cells differentiate to megakaryocytes in the marrow. Differentiated megakaryocytes extend proplatelets and translocate constituents of mature platelets to proplatelet tips in a highly regulated fashion. Platelets are "spawned" from proplatelet tips. Mature platelets then circulate in the blood for approximately 7 days, unless they are activated and deposited at sites of vascular injury, inflammation, and/or microvascular sequestration. There is evidence that thrombopoiesis occurs in the normal and injured lung. *See* text for details. (*B*) Key steps in thrombopoiesis are illustrated by photomicrographs from a model of proplatelet formation based on differentiation of $CD34^+$ human hematopoietic stem cell precursors isolated from umbilical cord blood. *See* Refs. 17 and 18 for details. Mature platelets freshly isolated from blood are shown in the *right-hand panel*. The photomicrographs are reprinted from Refs. 17 and 18 with permission.

subacute phase of ALI/ARDS. There is evidence that human megakaryocytes and a megakaryocytic cell line adhere to activated endothelial cells (28), which are present in macro- and microvessels in ALI/ARDS (29), providing a mechanism for targeting and localization of megakaryocytes to the injured pulmonary vascular bed. The molecular signals that might lead to migration and/or *in situ* differentiation of megakaryocytes in the injured lung are unknown, and it is also unknown if the injured lung provides organ-specific signals that alter thrombopoieis in the marrow. The number and activity of megakaryocytes (marrow, lung, or both), proplatelets, and platelets, and their contributions to pulmonary dysfunction and repair, are likely to be dynamic variables in the acute edematous phase versus the fibroproliferative phase of ALI/ARDS and in patients with resolving ALI (7, 30). These issues are also largely unexplored.

TRADITIONAL AND NONTRADITIONAL ROLES OF PLATELETS IN HEMOSTASIS AND INFLAMMATION RELEVANT TO ALI/ARDS

Platelets are the chief cellular effectors of hemostasis. A primary function of platelets, well known to clinicians and investigators alike, is to limit hemorrhage after trauma and vascular injury (reviewed in Refs. 3 and 31). The lung is a site of hemorrhage in thrombocytopenic patients. In addition, thrombocytopenia is a relative contraindication to lung biopsy and other invasive procedures. Bleeding and therapeutic hemostasis are daily issues in the ICU. Thus, practicing chest and critical care physicians consider hemostatic functions of platelets on a regular basis.

Platelets initially adhere to exposed subendothelial matrix at sites of endothelial disruption via the glycoprotein Ib/V/IX complex, which recognizes von Willebrand factor and other ligands (31). Several surface integrins and collagen receptors also tether platelets to matrix. Activation of platelets via G protein–coupled receptors that recognize thrombin, adenosine diphosphate, endogenously generated thromboxane A_2, and other agonists then rapidly amplifies the initial adhesion, and mediates aggregation and recruitment of additional platelets to the site of injury. Cellular activation converts integrin $\alpha_{IIB}\beta_3$ (glycoprotein IIb/IIIA), a major cell-specific integrin on megakaryocytes and platelets, to a state that binds fibrinogen, fibrin, and other ligands, mediating homotypic aggregation and additional adhesive events. Each of these responses occurs within seconds to minutes after activation. Genetic or pharmacologic interruption of one or more of these rapid steps causes bleeding, providing clear evidence for critical roles of platelets in physiologic hemostasis. Each step is a major target for current therapeutic strategies in thrombotic diseases (3, 31). In addition to rapid formation of a hemostastic barrier and provision of cellular components to the clot scaffold, platelets also mediate clot retraction. This is a more prolonged process that has largely been defined *in vitro*, and that is thought to stabilize clots and initiate their remodeling (3, 18).

Endothelial cells can regulate rapid hemostatic responses of platelets. At least three molecular systems contribute to the antithrombotic armamentarium of endothelium: cyclooxygenase/prostacyclin, L-arginine/nitric oxide, and ecto-adenosine diphosphatase (31). Because of the potency of these endothelial mechanisms as regulators of platelet activation and responsiveness, platelets were previously thought to deposit exclusively on exposed subendothelial structures and matrix at sites of primary hemostasis and clot formation, and to be repelled from endothelial surfaces. Exposed subendothelial matrix is clearly a favored site of platelet deposition in the flowing blood (32). Nevertheless, more recent observations, largely in rodent models, indicate that platelets can adhere to injured or inflamed endothelial cells (reviewed in Refs. 33 and 34). This may occur in the pulmonary circulation (35). Activated platelets that accumulate at sites of vascular injury or disruption can deliver signals that induce inflammatory responses of endothelial cells, a potential amplification mechanism in hemostasis, thrombosis, and inflammation.

Platelets have activities relevant to hemostasis beyond the traditional and well-known responses of adhesion, aggregation, hemostatic plug formation, and clot retraction at sites of vascular injury. Activated platelets provide a surface for catalysis of the extrinsic clotting cascade by the tissue factor/factor VII_a complex (36). This is critical in clot propagation and stabilization. Microparticles bearing tissue factor (TF) released from activated platelets or other cells may also be involved in clot propagation (36, 37). The sources of TF displayed by activated platelets and platelet microparticles have been controversial. TF-rich microparticles derived from stimulated monocytes can bind to activated platelets via PSGL-1 on the microparticle and P-selectin on the platelet (reviewed in Ref. 36). Recent observations, however, indicate that activated human platelets synthesize TF and may also be a primary source of this procoagulant protein in hemostasis and thrombosis (38, 39). TF synthesis by activated platelets is regulated by novel signal-dependent pre-mRNA splicing and translation pathways (38) (also *see* below). These observations have the potential to alter paradigms in the field because they suggest that TF may contribute to both initiation and amplification of the clotting cascade if it is rapidly synthesized and displayed by activated platelets at sites of primary hemostasis (36, 38). In parallel, there is evidence that activated platelets provide signals that induce TF synthesis by monocytes (40, 41), which also accumulate in clots and at sites of vascular injury (42 and references cited therein). Thus, activated platelets have recently identified activities that can influence clot formation via mechanisms that complement their rapid deposition in a hemostatic plug. These activities, or others such as inflammatory functions (*see below*), could provide new molecular targets in the treatment of venous thrombosis and thromboembolism (42) or acute lung injury (43).

While platelets are largely known for their immediate hemostatic activities, this is too restrictive a view of their functions in health and disease. They have nontraditional activities that, while secondary to clotting, nevertheless are tightly linked to hemostasis and to maintenance of tissue integrity (44). As a clear example, platelets are remarkably diverse inflammatory and immune effector cells, and they respond to, and deliver, signals that link inflammation and hemostasis (2, 21, 22, 33, 34, 45). This may be an evolutionarily conserved capacity that originated at an ancient time when host defense was accomplished by one, or a small number, of multifunctional cells with antimicrobial and wound repair activities; precursors of modern platelets then became progressively more specialized for hemostasis, but retained and evolved molecular systems for interacting with leukocytes and performing inflammatory and immune functions (2, 45). An important and relatively recent discovery that supports this possibility is the observation that mouse and human platelets have functional Toll-like receptors (TLRs) that recognize bacterial lipopolysaccharides and other microbial products (46–50). TLR expression presents a new facet in the repertoire by which platelets interact with, and respond to, microorganisms and their molecular signals, contributing to antimicrobial host defense (51). In addition, TLR signaling mediates inflammatory and thrombotic responses in sepsis and other pathologic conditions (52). Other signal transduction systems in platelets, such as those

activated by thrombin or PAF (3), also link hemostasis and inflammation under physiologic and pathologic conditions (52).

In addition to TLR signaling, platelets have other inflammatory capacities. They release a variety of preformed chemokines and cytokines, providing a mechanism for rapid contributions to acute inflammatory responses (45) (Figure 3). In addition, they rapidly synthesize the eicosanoid thromboxane A_2, which has immunomodulatory as well as hemostatic activities (53). Unexpectedly, platelets also synthesize interleukin-1β (IL-1β) (Figure 3) (17, 54), a pleiotropic inflammatory regulator (55) with multiple actions in lung injury and repair (56–59). IL-1β synthesized by activated platelets can induce inflammatory responses of human endothelial cells (Figure 3). Synthesis of IL-Iβ by activated human platelets occurs via signal-dependent pre-mRNA splicing and translation (*see below*).

Activated platelets interact directly with polymorphonuclear leukocytes (PMNs, neutrophils) and monocytes using P-selectin and other cell surface adhesion molecules and paracrine factors (2, 45). Physiologically relevant changes in function of the interacting cells result from these adhesive and signaling interactions. These include altered gene expression, with the potential to change patterns of expressed inflammatory and hemostastic factors in the local milieu (*see below*). Platelets also have the potential to directly or indirectly alter the ontogeny and function of macrophages and dendritic cells (45, 60–64). Dendritic cells are major effectors of "command and control" in

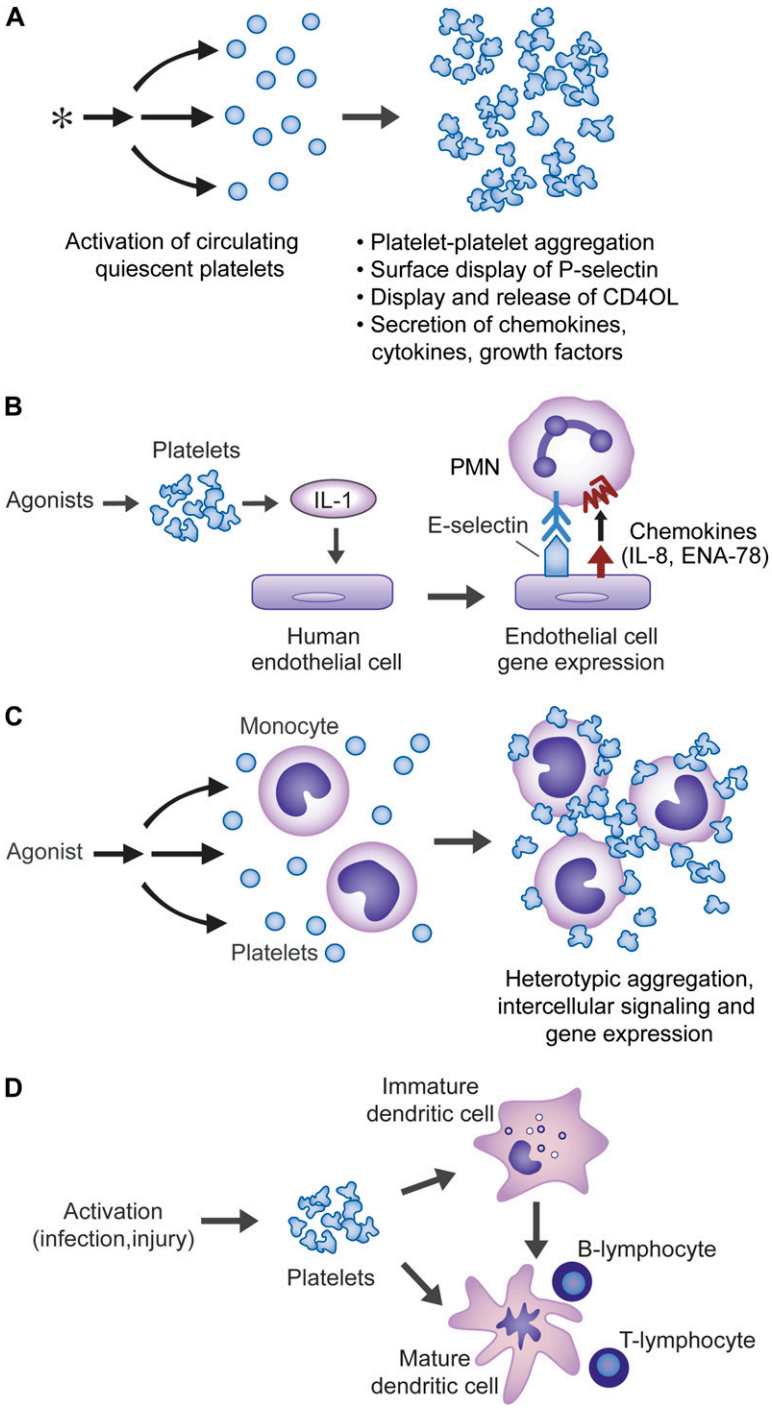

Figure 3. Platelets have activities that span the continuum from acute inflammation to adaptive immune responses. (*A*) Activated platelets can mediate inflammatory and immune responses using a variety of molecular mechanisms, including release of stored chemokines (β-thromboglobulin, platelet factor 4, ENA-78, Gro α, RANTES, SDF1α, and others), cytokines (HMBG1), and growth factors (PDGF, others). Activated platelets also synthesize lipid mediators including thromboxane A_2 and PAF, which have inflammatory and immune activities. The spectrum of inflammatory factors released from activated platelets is reviewed in Refs. 34 and 45. (*B*) In addition to lipid mediators, platelets rapidly synthesize IL-1β. This occurs via a regulated post-transcriptional pathway. IL-1β can then remain cell associated or be released in solution or in association with microvessicles. When synthesized by activated platelets *in vitro*, IL-1β is deposited in fibrin clots; if this occurs *in vivo*, clots and thrombi may act as local reservoirs for the cytokine. Platelet-derived IL-1β can signal human endothelial cells, leading to synthesis of adhesion molecules and chemokines that mediate PMN accumulation and activation (54). Platelets can also induce inflammatory responses by display of CD40 ligand (CD40L), which is recognized by CD40 on the surfaces of endothelial cells (34). (*C*) Activated platelets display P-selectin, which can mediate their adhesion to monocytes by binding to PSGL-1 on the leukocyte surfaces. P-selectin acts in concert with other adhesion molecules on the platelet plasma membrane and with chemokines and cytokines released by the platelets to mediate intercellular signaling and inflammatory gene expression by the target leukocytes. Activated platelets can also bind to PMNs (*see* Figure 4) and lymphocytes and alter their responses (reviewed in Refs. 2 and 45). (*D*) Activated platelets can signal functional changes in dendritic cells using multiple molecular pathways, and can potentially also influence differentiation of monocyte precursors to dendritic cells or macrophages (reviewed in Ref. 45). This figure was modified from Ref. 45 with permission.

interactions between innate and acquired immunity (65). They can also transition to macrophages (66). Thus platelets have molecular systems that influence the full spectrum of the inflammatory continuum, from innate to acquired immune responses (45) (Figure 3). Because of this, they have the capacity to alter events in a broad group of inflammatory alveolar, airway, and pulmonary vascular diseases.

PLATELETS IN CLINICAL ALI AND ARDS

Dysregulated hemostasis and inflammation are pivotal pathophysiologic mechanisms in ALI/ARDS, and have emerged as therapeutic targets in these syndromes (7, 30, 43). There is clinical evidence that platelets, as potent hemostatic and inflammatory cells, directly contribute to these central mechanisms.

Autopsy and biopsy studies of the lungs of patients with ALI/ARDS demonstrate intravascular microthrombi and fibrin deposition. In a study of 30 patients with ARDS, 23% developed disseminated intravascular coagulation (DIC), all of whom died (24). Autopsies were conducted in five of the seven fatal cases, and each revealed microthrombi. The lung was the most commonly involved organ, followed by the kidney and skin. Autopsies of patients with ARDS who did not develop overt DIC also revealed microthrombi in the lungs. The authors concluded that deposition of platelets on damaged pulmonary microvascular endothelium is common in ARDS regardless of whether or not it is accompanied by overt manifestations of the clinical and laboratory syndrome of DIC (24).

In a classic series of autopsy studies, Bachofen and Weibel reported platelets, intravascular fibrin deposits, and organized microthrombi in alveolar capillaries in the acute, exudative stage of ARDS (67), which is characterized by altered alveolar capillary membrane barrier function and increased pulmonary capillary permeability (7, 30). Morphometic analysis demonstrated increased numbers of platelets and leukocytes in lung capillaries. In some histologic samples platelets were apparently exiting pulmonary microvessels and entering the interstitial space (67). Subsequently, platelet–fibrin thrombi in pulmonary arteries, arterioles, and capillaries were detected in autopsy studies that used both microscopy and postmortem balloon angiography (68–70). Macrothrombi (in pulmonary arteries > 1 mm in diameter) were frequent in patients dying in the acute phase of ARDS and corresponded to vascular filling defects detected by bedside balloon occlusion pulmonary angiography in 48% of patients with ARDS (70). Although macrothrombi were particularly frequent in the acute phase, both macro- and microthrombi were present in all stages of the syndrome (68, 70). Open lung biopsy samples from patients with ALI also contained macro- and microthrombi (71). None of these autopsy or biopsy studies commented specifically on the presence of megakaryocytes (*see above*).

Consistent with morphologic observations, autologous radiolabeled platelets sequestered in the lungs, liver, and spleen when infused into patients with ALI (6, 25). Thus, there is anatomic and functional evidence demonstrating that platelets deposit in the acutely injured lung (8). The exact contributions of platelets to microthrombi formation, fibrin deposition, and thrombus remodeling in ALI/ARDS remain to be determined. Of interest, the influence of variables such as fraction of inspired oxygen (72) and protective versus damaging patterns of ventilation (30) on platelet accumulation in the injured human lung have not been defined.

As noted previously, thrombocytopenia occurs frequently in ALI/ARDS, with a variable incidence depending on the underlying clinical "trigger" (6). In addition to more common causes of diffuse alveolar damage such as trauma and sepsis (7), thrombocytopenia and ALI have also recently been associated with Severe Acute Respiratory Syndrome (SARS) and avian influenza A(H5N1) (73, 74). At the other end of the spectrum, thrombocytosis occurs in some patients with ALI/ARDS (*see above*). Interestingly, thrombocytosis, which also occurs in a variety of other inflammatory pulmonary syndromes, is a reported cause of pseudohypoxemia. The apparent impact of the circulating platelet number on oxygen exchange in subjects with septic shock or severe community-acquired pneumonia, which are important underlying conditions that contribute to ALA/ARDS, appears to be variable (F. Bozza and colleagues, unpublished observations).

In addition to alterations in circulating platelet number, there is evidence for altered phenotype and activation state of these cells in ALI/ARDS (6, 9). Circulating platelets from subjects with ARDS were reported to display P-selectin (formerly called GMP-140), a marker of activation, on their surfaces (75). P-selectin on activated platelets mediates adhesive interactions with neutrophils and monocytes (*see below*). PMN adhesiveness in pulmonary artery blood samples from patients with ARDS was influenced by platelet number (26), suggesting that platelet activation and altered platelet–leukocyte interactions may determine sequestration of these cells and other adhesive events in pulmonary vascular beds. Specific receptors on platelets from subjects with sepsis, one of the most common causes of ALI/ARDS (7, 30), were occupied by platelet-activating factor (PAF) (76), an agonist implicated in sepsis-induced ALI (52). Systemic and/or local intrapulmonary generation of platelet agonists such as thrombin, PAF, and other factors would provide mechanisms for platelet activation, increased adhesiveness, and additional functional responses such as degranulation in conditions that underlie ALI/ARDS (Figures 1, 3, and 4). Consistent with the latter possibility, bronchoalveolar lavage (BAL) samples from subjects with ARDS contain markers of platelet activation and release, but the levels do not clearly distinguish them from samples collected from subjects with interstitial lung disease or congestive heart failure (77, 78). Beta thromboglobulin, a platelet chemokine, was detected in the blood of patients with ALI and subjects with predisposing conditions without respiratory failure (79).

Recent studies of transfusion-related acute lung injury (TRALI) provide topical observations relevant to platelet participation in ALI. TRALI is a syndrome of noncardiogenic pulmonary edema that is temporally related to transfusion of blood products, usually occurring within 30 minutes to 6 hours of their administration (80). All plasma-containing blood products have been associated with TRALI, including platelet transfusions (80). Clinical and experimental studies indicate that TRALI is caused by infused antineutrophil antibodies and/or biologically active lipids in the plasma of the transfusate, acting together with "primed," adherent PMNs (80–82). Stored platelet concentrates are a source of lipids that can induce ALI in rats pretreated with LPS (82), consistent with this concept. It is not clear if transfused platelets themselves and/or endogenous activated platelets in the circulation of patients with TRALI play direct roles in the lung injury, although recent animal models suggest potential mechanisms (*see below*). If PMNs are activated by antineutrophil antibodies (81) and/or platelet-derived lipid mediators (82), they can then potentially activate platelets (reviewed in Refs. 6 and 83) contributing to alveolar capillary membrane injury. In an experimental model, infusion of a neutrophil agonist induced neutropenia, thrombocytopenia, and platelet sequestration in the lung (84), consistent with this possibility.

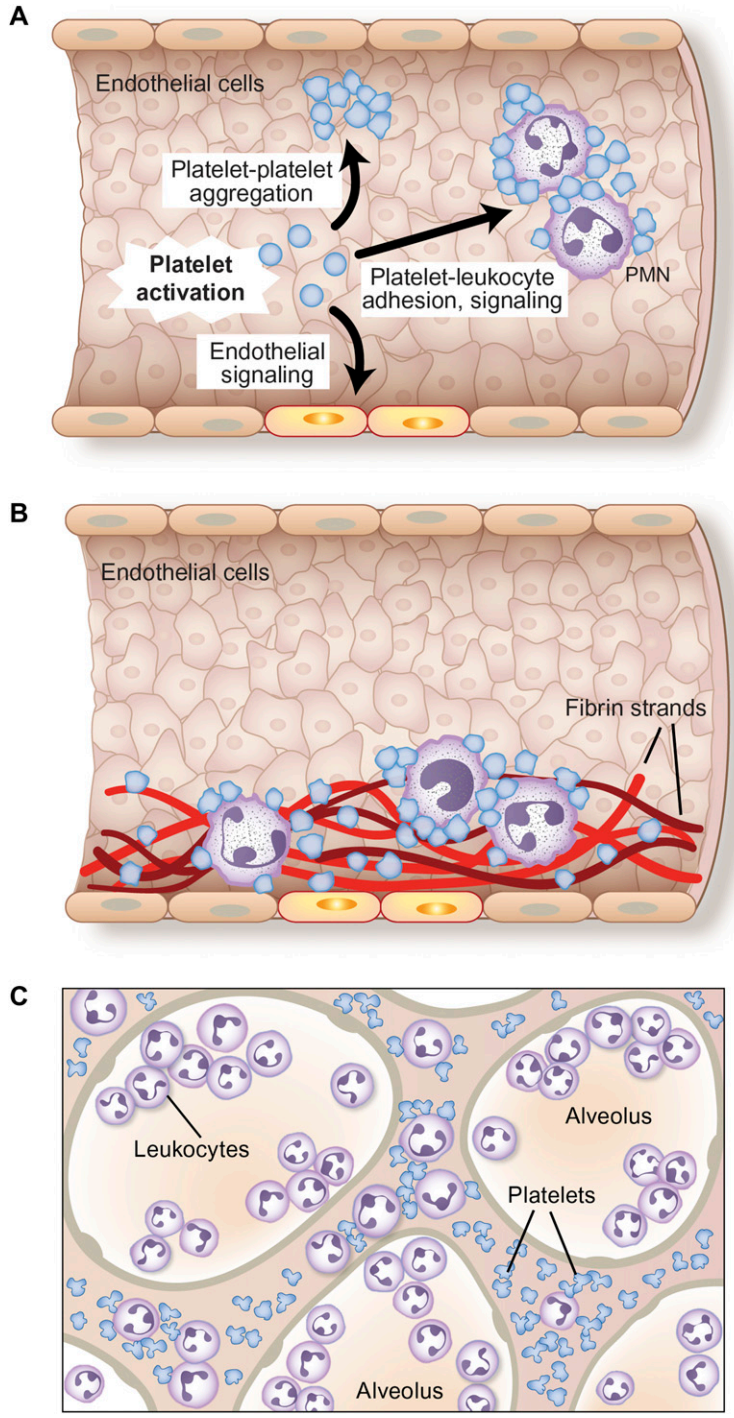

Figure 4. Platelet–PMN interactions influence acute lung injury in patients and experimental models. (*A*) When platelets are activated by prothrombotic and inflammatory signals, platelet–platelet, platelet–endothelial, and platelet–PMN interactions occur in pulmonary arterioles, capillaries, and venules. Several molecular interactions mediate adhesion of activated human and/or murine platelets to PMNs. These include P-selectin/PSGL-1; integrin $\alpha_m\beta_2$ (MAC-1) on the PMN interacting with integrin $\alpha_{IIb}\beta_3$ or ICAM-2 on the platelet with fibrinogen as an intermediate; integrin $\alpha_m\beta_2$ on the PMN binding to GPIbα or junctional adhesion molecular 3 on the platelet; ICAM-2 on the platelet binding to integrin $\alpha_L\beta_2$ (LFA-1) on the PMN (reviewed in Ref. 34). Platelets also interact with monocytes under these conditions (*see* Figure 3). These events likely contribute to physiologic responses in lung infection and trauma, but can also mediate acute lung injury. (*B*) Platelets and PMNs accumulate in macro- and microvessels in the lungs of patients with ALI/ARDS, and lungs of animals in models of ALI. Sequestration of platelets and PMNs is frequently associated with fibrin deposition and endothelial injury. (C) In mouse models of ALI, platelets facilitate accumulation of PMNs in pulmonary capillaries and alveolar spaces, and contribute to alveolar-capillary membrane injury. Platelet–PMN interactions mediated by P-selectin/PSGL-1 are involved, as are other molecular and signaling pathways (*see* text for details).

SYNTHETIC FUNCTIONS OF ACTIVATED PLATELETS: KNOWN PATHWAYS AND NEW BIOLOGY

Platelets rapidly synthesize thromboxane A_2 from arachidonic acid via the cyclooxygenase pathway, and also use 12 lipoxygenase to metabolize arachidonate to 12- hydroxy–eicosanoids (6, 21). Thromboxane A_2 has prothrombotic and proinflammatory activities, as previously outlined, and platelet 12 lipoxygenase can mediate transcellular metabolism and production of lipoxins in platelet–neutrophil interactions (21). Platelets also synthesize PAF (3). In addition to lipids, activated platelets produce superoxide and other reactive oxygen species (ROS) via NADPH oxidase activity (31). The generation of superoxide by activated platelets enhances their accumulation in growing thrombi and may impair the inhibitory activities of NO locally released by endothelial cells (31). The capacity to synthesize thromboxane, ROS, and lipoxins provides mechanisms by which activated platelets can modify vasoactive, inflammatory, and hemostatic events in ALI/ARDS (6, 85).

While synthesis of biologically active lipids and ROS are established functions of activated platelets, it has only recently been discovered that mature, circulating platelets synthesize proteins in response to thrombin and other physiologically relevant activating signals. This *de novo* protein synthesis occurs via a repertoire of post-transcriptional pathways, consistent with the fact that platelets are anucleate and do not transcribe messen-

ger mRNAs (with the exception of mitochondrial transcription). The general term assigned to synthesis of specific proteins by activated, but not resting, platelets is *signal-dependent translation* (reviewed in Ref. 44). The first example was synthesis of B cell lymphoma-3 (BCL-3) by activated human platelets, a process that is under specialized translational control by the intracellular kinase mammalian target of Rapamycin (mTOR) (86) (reviewed in Ref. 44). Synthesis of Bcl-3 by activated human and murine platelets regulates clot retraction in *ex vivo* assays (18), demonstrating that signal-dependent translation of previously transcribed but basally "silenced" mRNAs by activated platelets has physiologic relevance (44). An important point is that signal-dependent synthesis of Bcl-3 and other key proteins is rapidly initiated (86). Expedited synthesis of the protein products is a biologic advantage of translational control of previously transcribed mRNAs, and is one that is clearly advantageous to platelets since they are rapid response cells in injury and hemostasis. In addition, however, signal-dependent translation of new proteins by activated human platelets continues for several hours, indicating that platelet responses are not limited to adhesion, aggregation, and degranulation in the first few minutes of hemostasis (44, 45).

As noted above, activated human platelets also synthesize IL-1β and TF (17, 38, 39, 54). Remarkably, in both of these cases intron-containing pre-mRNA transcripts for each gene product are present in resting, unstimulated platelets and are spliced to mature, translatable mRNAs in a signal-dependent fashion in response to cellular activation (17, 38). Extranuclear splicing had not previously been observed in human cells before these observations. Based on studies of model megakaryocytes, the pre-mRNAs are translocated to proplatelets during thrombopoiesis (17, 38). In response to signals that induce splicing, activated platelets then translate the processed, mature mRNAs to biologically active TF, which mediates a procoagulant platelet surface, and to pro–IL-1β, a portion of which can be converted to active IL-1β (17, 38, 54). TF and IL-1β have many established and postulated activities in experimental and clinical ALI; therefore, their synthesis by activated platelets has the potential to influence key events in this setting. In addition, it is possible that signal-dependent translation and synthesis of Bcl-3 by activated platelets (18, 87) may modify fibrin deposits and intravascular thrombi in the injured lung (43) (Figure 4). Other products in the proteome of activated human platelets have also been reported to be synthesized in response to cellular stimulation, and many newly synthesized proteins are yet to be identified (45, 87). Some of these may have pathophysiologically relevant activities in acute lung injury syndromes.

In addition to acting as templates for new protein synthesis, the transcriptome of platelets from subjects with ALI/ARDS may provide unique diagnostic information. In a recent report, analysis of platelets from patients with myocardial infarction revealed differences compared with the mRNA profile in platelets from subjects with stable coronary artery disease (88). Similar analysis may be informative and useful in samples from subjects with ALI/ARDS and key predisposing conditions such as sepsis, in which platelet activation is frequent and may be ubiquitous (89).

PLATELETS, ENDOTHELIAL CELLS, AND ALVEOLAR CAPILLARY MEMBRANE PERMEABILITY

Platelets can interact with and signal endothelial cells in a variety of ways (6, 34, 90). In this section we focus on interactions that influence alveolar capillary barrier function because of its central importance in ALI/ARDS, particularly in the early edematous phase (7, 30).

Clinical and experimental observations indicate that platelets are important, and potentially essential, for systemic and pulmonary vascular integrity. As an example, thrombocytopenia is associated with pleural effusion—which was shown to be a measure of vascular leakage—in Dengue syndromes (91). Extensive evidence for a contribution by platelets to basal vascular integrity has been reviewed (6). It includes the observation that infusion of small numbers of platelets insufficient to alter the bleeding time improved vascular fragility in patients with leukemia. In studies of systemic tissues, vascular permeability increased when experimental animals were made thrombocytopenic, and perfusion of isolated organs with platelets corrected ultrastructural alterations in endothelium associated with altered barrier function. Studies of the lungs in both large and small animal models indicated that platelets contribute to pulmonary vascular integrity and to alveolar–capillary barrier function. Platelets were proposed to take up and eliminate noxious barrier-disrupting agents such as ROS, to plug paracellular gaps, and/or to provide factors that decrease endothelial permeability and stimulate endothelial survival and barrier function. Experimental evidence suggested that serotonin, epinephrine, and adenosine released from activated platelets induce endothelial stress fiber organization and/or replication of endothelial cells, depending on the specific factor studied. These and other observations indicated that platelets are critical for vascular barrier function; nevertheless, the conclusion was that the mechanisms by which platelets preserve vascular integrity remained elusive (6).

More recent studies have identified several phospholipids with signaling properties for endothelial cells that are released from platelets (92, and references cited therein). One of these, sphingosine-1-phosphate (S1P), was reported to promote endothelial barrier integrity *in vitro* and *in vivo*, including improvement of barrier function in models of increased pulmonary capillary permeability. S1P is recognized by a family of five surface G protein–coupled SIP receptors, which are also called Edg receptors (92). S1P released from activated platelets was shown to interact with endothelial cells (93), and to induce adherens junction assembly and cytoskeletal rearrangement in an *in vitro* model of angiogenesis using cultured human umbilical vein endothelial cells (94). Adherens junctions and tight junctions are critical in regulating endothelial permeability and polarity, and can be modified by inflammatory mediators and hemostatic signals in pulmonary vascular injury (29). S1P induced rapid and sustained increases in transmonolayer electrical resistance in cultured human and bovine pulmonary artery and microvascular endothelial cells by engaging S1P receptors (92), paralleling improved barrier function in "leaky" human umbilical vein endothelial cells in the angiogenesis model (94). Reduction in S1P receptor Type 1 ($S1PR_1$) by selective "knockdown" using RNA interference altered human endothelial cell function and modified parallel TNF-α signaling (95). In addition, endothelial barrier function was reported to be improved by activated protein C, which is used therapeutically in the treatment of sepsis, by a mechanism that involves transactivation of $S1PR_1$ by protease-activated receptor 1 (96).

Exogenous administration of S1P was later shown to incrementally reduce pulmonary microvascular permeability and alter indices of alveolar inflammation in mice and dogs subjected to LPS challenge and in a model of ventilator-induced lung injury (97, 98). Administration of S1P also reduced extravasation of labeled albumin and accumulation of myeloperoxidase, a PMN marker, in the kidneys of LPS-infused mice in these experiments. In aggregate together with *in vitro* studies, these observations suggested that S1P may be useful as a therapeutic agent in syndromes of vascular barrier dysfunction (99), and also provided a previously unrecognized mechanism by

which platelets may contribute to vascular integrity. Importantly, steady-state plasma levels of S1P are significantly higher than the K_d for SIP receptors, suggesting that endothelial cell S1P receptors are tonically ligated by endogenous S1P under physiologic conditions (99). Nevertheless, this may not be the case in thrombocytopenic patients. In addition to clinical issues of this sort, a number of mechanistic and physiologic issues regarding the activities of S1P in altered lung barrier function remain to be resolved (99).

It is not clear if platelets also influence alveolar epithelial function. This question is important because epithelial cells, together with endothelial cells, are critical in regulation of alveolar capillary membrane permeability and alveolar fluid clearance (30, 100). Because platelet products are found in the alveolar fluid in ALI (*see above*), activated platelets may deliver paracrine signals such as IL-1β or other soluble inflammatory factors (34, 45) to the alveolar epithelium.

In addition to releasing molecular factors that provide direct signals to alveolar capillary endothelial or epithelial cells, platelets may alter alveolar barrier function by interacting with leukocytes. Examples in the next section illustrate this point.

PLATELETS, LEUKOCYTES, AND ALI

As emphasized previously, platelets have molecular systems and functional activities that link hemostasis and inflammation. Platelet–leukocyte interactions are central in this repertoire (Figures 3 and 4). Activated platelets adhere to myeloid leukocytes in the circulating blood and in microvascular beds, forming heterotypic aggregates (2, 45). In some clinical conditions detection of circulating platelet–monocyte aggregates by flow cytometry may be the most sensitive measure of platelet activation (101). Activated platelets adhere to monocytes and neutrophils (Figures 3 and 4) by binding of P-selectin, which is translocated from α granules to the platelet plasma membrane, to P-selectin glycoprotein ligand 1 (PSGL-1) on the leukocyte surfaces (reviewed in Refs. 34 and 45). Integrin $\alpha_{11b}\beta_3$ and glycoprotein Ib on activated platelets, together with integrin $\alpha_m\beta_2$ (MAC-1, CD11b/CD18) on the myeloid leukocytes, also contribute to these adhesive interactions (34, 102, 103). Platelets facilitate recruitment of PMNs to the surfaces of inflamed endothelial cells in experimental models (104). In addition, adherent, activated platelets deposited on exposed vascular matrix provide a surface for rolling and attachment of myeloid leukocytes in experimental studies (2, 34). Whether this occurs in the human lung vessels is unknown. In an experimental model, PMNs sequestered in lung microvessels facilitated deposition of platelets, suggesting that neutrophil–platelet interactions influence the local deposition of platelets in alveolar inflammation (84).

Adhesion of activated platelets to PMNs and monocytes is more than a physical association. Platelets also provide juxtacrine and paracrine signals that can induce functional alterations in the leukocytes (6, 83, 105). As an example, activated platelets release the chemokine RANTES, which can deliver signals to monocytes via CC chemokine receptors on their surfaces (106). In addition, PSGL-1 on monocytes and PMNs can transmit juxtacrine signals when it is engaged by P-selectin on activated platelets (2, 105). One consequence of signaling of myeloid leukocytes by activated platelets is the expression of genes that encode inflammatory proteins and enzymes, including IL-8, monocyte chemoattractant protein-1, cyclooxygenase-2, and others (2, 105, 107). There is also evidence for reciprocal signaling between activated platelets and leukocytes and, as mentioned previously, transcellular metabolism (6, 83). These processes are influenced by cell–cell adhesion and signaling mediated by P-selectin, other adhesion molecules, and soluble factors.

Platelet interactions with myeloid leukocytes were previously implicated in ALI/ARDS based on a variety of observations (6, 8). In addition, platelets are found together with PMNs and monocytes in lung vessels in histologic samples from subjects with ALI/ARDS (*see above*). Early laboratory studies suggested a role for platelets in acute lung injury; for example, platelets were reported to be required for complement-mediated ALI in mice (108). Recent experimental studies add to the body of evidence indicating that platelet–leukocyte interactions are important in ALI syndromes.

A murine model of lung injury induced by hydrochloric acid (HCL), used as a surrogate for human ALI/ARDS triggered by aspiration, indicated that platelet–PMN interactions are determinants of leukocyte accumulation, increased alveolar–capillary permeability, and impaired gas exchange (109). Platelet–neutrophil aggregates were detected in the blood and in lung microvessels within 30 minutes of acid injury. Platelet depletion reduced PMN accumulation in microvascular, interstitial, and alveolar compartments, an experimental finding with potential clinical correlates, since adhesiveness of PMNs was related to platelet numbers in pulmonary blood samples from patients with ARDS (26). Of note, engagement of PSGL-1 on human PMNs triggers release of IL-8 (110). IL-8 mediates PMN accumulation and alveolar capillary membrane injury in experimental acid aspiration (111).

Platelet depletion reduced the total protein recovered in BAL samples and improved the Pa_{O_2}/FI_{O_2} ratio in HLC-treated mice compared with those with normal platelet counts and, in limited experiments, improved these variables in a "two hit" model of sepsis (109). In addition, an anti–P-selectin mAb administered 15 minutes after acid injury also reduced PMN accumulation and protein content in alveolar fluid, and improved the Pa_{O_2}/FI_{O_2} ratio and ultimate survival. These results were, in general, consistent with previous studies indicating that blockade or knockout of P-selectin often protects animals from, or improves, inflammatory damage in models of ALI (104). Studies of chimeric mice indicated that P-selectin expressed by platelets—rather than by endothelial cells—was largely responsible for accumulation of PMNs, altered alveolar barrier function, and impaired gas exchange resulting from acid injury, although endothelial P-selectin contributed to intravascular PMN sequestration (109). Additional experiments using acid-challenged animals, cultured microvascular endothelial cells, and human platelets and PMNs identified thromboxane synthesis mediated by platelet–PMN interactions and signaling via thromboxane receptors as central components in this model.

Platelets were also reported to contribute to inflammatory lung injury in murine models of other syndromes, including hemorrhagic shock. Platelet depletion reduced infiltration of PMNs in the lungs of mice subjected to hemorrhage, and improved lung edema assessed by histologic measurements (112). In the same study, similar improvements in liver inflammation were seen in animals given an anti-platelet antibody before hemorrhage shock. Importantly, some but not all anti-platelet antibodies induce systemic changes and lung injury in mice (113), indicating that the method and timing of platelet depletion are important factors in these experimental models. In earlier large animal models (dogs, sheep), platelet depletion with anti-platelet sera had variable effects on pulmonary responses to LPS infusion (114, 115).

Observations suggesting that platelets are central effectors of experimental alveolar injury (108, 109, 112) pose a potential quandary when considered in the context of studies indicating that platelets are essential for normal alveolar capillary membrane integrity and that platelet products improve alveolar barrier function (discussed above). It is possible that negative

and injurious effects mediated by platelet–PMN interactions under conditions of injury (109) outweigh positive signals delivered to the pulmonary alveolar barrier by circulating platelets alone. PMNs activated by platelets and/or by other signaling pathways after being locally recruited by platelets have potent mechanisms that can increase vascular permeability and injure endothelial and epithelial cells (6, 116–118). Furthermore, it is possible that local release of S1P or other factors that stabilize barrier function by platelets is impaired under some injury conditions. With respect to contributions to basal barrier integrity, reduction of circulating platelets by approximately 40% caused a small but nonsignificant decrease in Pa_{O_2}/FI_{O_2} ratio in control mice (109), suggesting that this degree of platelet depletion did not alter alveolar barrier function; direct measurements of lung water and barrier properties were not reported, however. Beneficial effects of platelet reduction in the face of acid injury were durable when profound thrombocytopenia was induced in this model ($\sim$ 85% reduction), but the impact of this degree of platelet depletion on lung water and barrier function in control mice was again not investigated.

There are additional complex platelet–PMN interactions in models relevant to ALI/ARDS. Studies employing isolated human cells and a mouse model of endotoxemia indicated that, under conditions chosen to be relevant to sepsis, platelets induce formation of neutrophil extracellular traps (NETs) (119). NETs are extracellular chromatin lattices studded with elastase and other antimicrobial factors that capture and kill bacteria and fungi (120). This is a previously unrecognized mechanism of extracellular antimicrobial defense (121). In flow chamber experiments using human cells, LPS-treated platelets adhered to PMNs immobilized on coverslips and induced NET formation (119). This could also be triggered by plasma by from patients with sepsis, but not by thrombin. This, and features of the adhesive interaction between platelets and PMNs under these conditions, suggest an unconventional mechanism of platelet activation mediated by TLR4. In mice infused with LPS, platelet–PMN interaction occurred in liver sinusoids and pulmonary capillaries and NET-like structures were detected in pulmonary vessels. In the liver, NET formation was associated with evidence of microvascular injury that was prevented by prior depletion of platelets or neutrophils. This, together with experiments using cultured endothelial cells, suggested that platelet-triggered NET formation causes endothelial damage if it occurs in the microcirculation, and that platelet-induced NET generation may be a cause of microvascular dysfunction in sepsis (119, 121). The exact mechanism of endothelial injury was not examined, leaving this question open. NETs have not been observed in lungs or systemic vessels of patients with sepsis, but they have been reported in appendicitis, in the alveolar space in bacterial pneumonia, and in interstitial pneumonia in tissues from human subjects (119, 120, 122).

In considering the mechanisms involved in murine ALI models in the context of clinical ALI/ARDS, it should be remembered that the number of circulating platelets is higher and the number of PMNs lower in mice compared with humans (20, 123). Therefore, the platelet/PMN ratio in systemic and pulmonary blood is substantially different in the two species. In the recent HCL injury model, the ratio in systemic blood of control mice was approximately 250/1 (109), whereas in human systemic blood it is approximately 80–100/1 under basal conditions.

PLATELETS AND REPAIR: INTERACTIONS WITH PROGENITOR AND STEM CELLS

Platelets have the potential to influence repair of alveolar and airway structures, and also to participate in maladaptive repair responses such as fibrosis, by cellular interactions and by releasing paracrine factors. These include platelet-derived growth factor, epidermal growth factor, vascular endothelial growth factor, and members of the transforming growth factor family (reviewed in Refs. 6 and 90). IL-1β and eicosanoids, which are synthesized by activated platelets and by monocytes in response to platelet signals (17, 54, 105, 107), also regulate responses of fibroblasts that are relevant to the fibroproliferative phase of AL1/ARDS (124). Thus, signaling interactions may allow platelets to influence homeostatic repair or, conversely, drive untoward cellular proliferation and fibrosis in the acutely injured lung.

Platelets may also influence repair by interacting with stem and progenitor cells. As an example, platelets were recently reported to bind to bone marrow–derived endothelial progenitor cells and to recruit them to thrombi and sites of vascular injury in murine models (125). Activated platelets adhered to progenitor cells using P-selectin and integrins, and also released stromal cell–derived factor 1α (SDF 1α), which further supported homing and adhesion of progenitor cells. Adherent platelets and fibrin were also reported to induce differentiation of progenitor cells to a phenotype that expressed endothelial markers in an *in vitro* model (126). In a different murine model, hematopoietic cytokines were reported to induce local "deployment" of SDF 1α from platelets and, via this mechanism, mediate mobilization and recruitment of precursor hemangiocytes to ischemic hindlimb vessels (127). Thus, platelets may have key regulatory activities that are critical in endothelial precursor homing and differentiation and in vascular repair (reviewed in Ref. 128). Whether platelet-mediated fibrin modulation (18) and/or deposition of platelet factors such as IL-1β in the fibrin reservoir (54) at sites of injury condition the local niche and favor endothelial precursor cell homing and differentiation are unknown.

Platelet-directed progenitor cell recruitment may drive vascular injury in addition to mediating vascular repair and development. Platelet signaling is reported to direct progenitor cells along differentiation pathways that lead to foam cell formation under some conditions (128). Foam cell formation is considered to be a maladaptive response in atherosclerosis.

While platelet interactions with endothelial progenitor cells have largely been considered in the context of atherosclerosis and ischemic peripheral vascular injury to date, it is possible that these observations are also relevant to repair versus obstruction and destruction of injured pulmonary vessels. Dysregulated vascular remodeling can be severe in the acutely injured lung, and is a cause of vascular obliteration and pulmonary hypertension in ALI/ARDS (30, 68, 70). The contributions of platelets and their interactions with progenitor cells in pulmonary vascular repair and remodeling in ALI/ARDS are unexplored. It is also unknown if platelets influence homing and/or differentiation of bone marrow–derived mesenchymal stem cells to the injured lung (129), or targeting and differentiation of specific progenitor cell types to the kidney or other damaged tissues in multiple organ injury in the setting of ALI/ARDS. In addition, platelets themselves—engineered from hematopoietic stem cells—could be unique vehicles for delivery of reparative factors in cell-based therapeutic strategies (130). These possibilities are intriguing and would provide new facets to the activities of platelets in host injury, defense, and repair.

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