

The role of platelets in acute lung injury (ALI)

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1. ABSTRACT

Acute lung injury (ALI) is a common syndrome associated with a high mortality rate. Better understanding of the pathophysiology of acute lung injury and progress in supportive care and mechanical ventilation have led to slightly improved clinical outcomes. New evidence shows that the interplay between platelets, leukocytes and endothelial cells is critical in the pathogenesis of ALI. Key molecules involved in this interaction include P-selectin and the eicosanoid thromboxane A₂ (TXA₂), suggesting potential new targets for pharmacological intervention. In this review, we summarize the aspects of the interactions between platelets, leukocytes, and endothelial cells that are relevant for the pathogenesis of ALI.

2. INTRODUCTION

2.1. Definition, epidemiology

Acute lung injury (ALI) is a common, devastating syndrome that affects surgical as well as medical patients. The syndrome is defined as an acute hypoxemic respiratory failure with bilateral pulmonary infiltrates that are not caused by left atrial hypertension (1). This new definition of ALI has the advantage of identifying patients at an early stage of the syndrome, but it does not consider the underlying cause of ALI or other affected organ systems that can influence the outcome. A recent study showed that the overall incidence of acute lung injury is 78.9 per 100,000 person-years in the United States (2). However, the incidence is age-dependent and increases

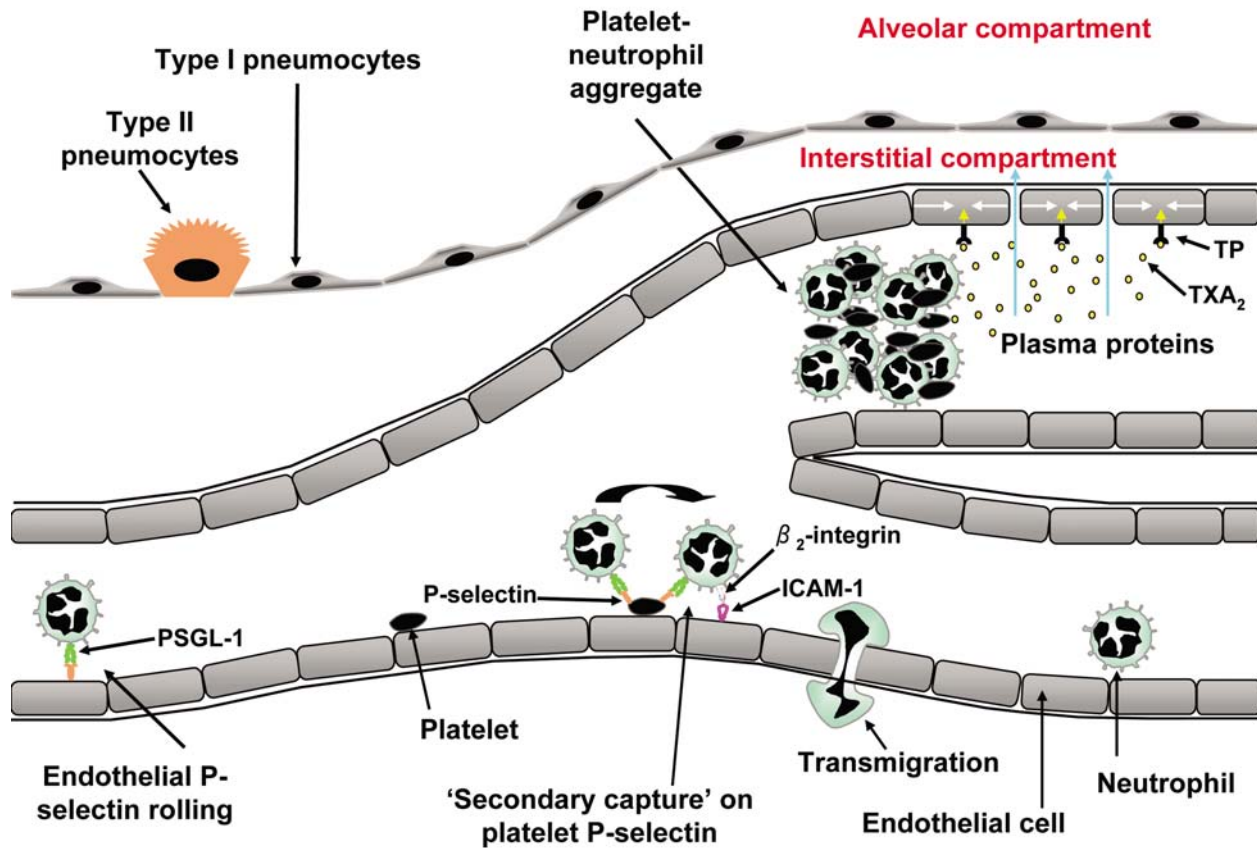


Figure 1. Role of platelets for neutrophil recruitment and regulation of vascular permeability in the lung. Neutrophils can roll on P-selectin expressed on endothelial cells (bottom left) and interact with platelets adherent to the endothelium (bottom center). This interaction is mediated by platelet P-selectin and PSGL-1 on neutrophils. This 'secondary capturing' might activate β_2 -integrins on neutrophils that subsequently can interact with ICAM-1 on endothelial cells (middle). Platelet-neutrophil aggregates (top right) lead to a reciprocal activation of the cells with a release of the lipid mediator thromboxane A_2 (TXA $_2$). TXA $_2$ binds to thromboxane receptors (TP) expressed on endothelial cells. The activation of the receptors leads to an increase of vascular permeability with subsequent egression of fluid and plasma proteins from the intravascular into the interstitial and alveolar compartments.

from 16 per 100,000 person-years for those 15 through 19 years of age to 306 per 100,000 for those 75 through 84 years of age (2). Despite improved treatment, the disease is associated with a high mortality of up to 38%. Based on these data, 190,600 cases of ALI occur every year in the United States and cause 3.6 million hospital days (2), which places a significant burden on the health care system.

2.2. Pathogenesis

The underlying causes of ALI can be divided into those associated with direct injury to the lung, including trauma, pneumonia, aspiration of gastric content, inhalational injury, reperfusion-induced pulmonary edema, and those that are caused indirectly in the context of systemic processes like sepsis, severe trauma with shock or multiple transfusions. The risk of developing ALI substantially increases in the presence of multiple predisposing disorders (3), including chronic lung diseases (4).

During the acute phase of ALI, the alveolar-capillary barrier, composed of vascular endothelium, a narrow interstitial space and alveolar epithelium, is damaged (Figure 1). This causes an increase of vascular and epithelial permeability with influx of protein-rich fluid into the interstitial and alveolar compartments (5), causing impaired gas exchange. This phase is characterized by neutrophil, macrophage, and erythrocyte infiltration and the formation of hyaline membranes (6). The damage to endothelial and epithelial integrity is of critical importance in ALI, as this barrier regulates permeability (7). Fluid transport is also regulated by the alveolar epithelium (8, 9), which is also involved in surfactant production (10). The reduced surfactant production caused by injury to alveolar type II pneumocytes leads to the formation of atelectasis (6).

ALI induces the production and release of a myriad of cytokines and other pro-inflammatory mediators. These mediators can initiate and amplify the inflammatory

response in ALI. Cytokines can be produced locally in the lung by alveolar macrophages, epithelial cells, fibroblasts, endothelial cells as well as by neutrophils and other leukocytes. The chemokine receptor CXCR2 is critically involved in the pathogenesis of different models of ALI (11-14). This chemokine receptor binds the chemokine CXCL1, 2, 3, 5, 6, 7, and 8 in humans and CXCL1, 2, 3, 5, 6, and 7 in mice. The receptor is involved in regulation of vascular permeability and neutrophil recruitment in different models of ALI (11-14). Other chemokines and pro-inflammatory mediators like CCL2 (Monocyte Chemoattractant Protein (MCP)-1) (15), cytosolic phospholipase A2 (cPLA2) (16), and platelet-activating factor (17) are also involved in regulating leukocyte recruitment and vascular permeability in the lung.

New evidence demonstrates that activated coagulation and impaired fibrinolysis are associated with ALI (18). During ALI, the coagulation system is activated, producing fibrin deposition in the lung (19, 20). Tissue factor (TF) released by activated monocytes binds coagulation factor VII, activates it to VIIa, which in turn converts X to its activated form Xa. This complex can produce thrombin and activate protease-activated receptors (PARs) on endothelial cells, inducing an inflammatory response with up-regulation of cytokines as well as thrombin formation. Thrombin also activates platelets by binding to platelet PAR1 and 4 (21) and induces the conversion of fibrinogen to fibrin that, together with activated platelets, can induce the formation of microvascular thrombosis. The blockade of TF by a monoclonal antibody or a site-inactivated factor VIIa in the context of an E.coli-induced sepsis reduced systemic inflammation, improved gas exchange and lung compliance, prevented fibrinogen depletion, and mitigated lung injury (22, 23). Lung-protective ventilation, which is associated with diminished release of pro-inflammatory mediators (24), also leads to reduced activation of the coagulation cascade (25).

Previous studies have shown that modulating the coagulation system by protein C can be beneficial in ALI (26). In a clinical study with patients suffering from septic and non-septic ALI, lower plasma protein C levels and higher levels of thrombomodulin in the bronchoalveolar lavage were associated with worse clinical outcomes, including death, fewer ventilator-free days, and more nonpulmonary organ failures (26). The alveolar epithelium expresses thrombomodulin and endothelial protein C receptor (EPCR), which may limit alveolar fibrin deposition (27).

2.3. Leukocyte recruitment into the lung

Neutrophil influx is a hallmark of ALI. Several clinical and experimental studies have demonstrated the importance of neutrophil-mediated injury during the development of ALI (28), but the exact recruitment mechanisms remain to be fully determined. Leukocyte recruitment in the systemic circulation proceeds in a multi-step cascade and is well established (29, 30), whereas the recruitment of leukocytes into the lung is influenced by complex processes including the unique capillary structure

of the lung, neutrophil deformability, and adhesion molecules (31). Due to the relation of the size of pulmonary capillaries (diameter 2-15 μm) (32) to the size of neutrophils, neutrophils have to stop several times and change their shape in order to negotiate the pulmonary capillaries. This leads to a significantly prolonged transit time compared to erythrocytes and a 40- to 100-fold neutrophil accumulation ('marginated pool') in the lungs (33).

In response to inflammatory stimuli, pro-inflammatory mediators are released that are able to activate neutrophils and change their biomechanical properties by promoting polymerization of subcortical actin with a subsequent sequestration of stiff neutrophils in the pulmonary microvasculature (34, 35). The role of adhesion molecules in the sequestration step is not fully understood. It has been shown that L-selectin, which is expressed on leukocytes, is required for the sequestration of neutrophils in the lung in response to the formyl peptide fMLP, but not C5a, as shown by using blocking antibodies and L-selectin-deficient mice (36). Blocking E-selectin and L-selectin by antibodies did not influence neutrophil sequestration in a model of sepsis-induced ALI (37).

The pulmonary capillary vasculature is the location of neutrophil migration. Neutrophils can migrate through the endothelium by penetrating interendothelial junctions or using a transcellular route (38). The interactions between neutrophils and vascular endothelium as well as the release of pro-inflammatory mediators by neutrophils induce cytoskeletal changes in the endothelial cells. Cell-cell-interactions lead to intracellular signaling through transmembrane proteins in the area of interendothelial junctions (e.g. platelet endothelial cell adhesion molecule (PECAM)-1, CD99) and this might trigger transient remodeling of the junction (39-41).

Dependent on the stimulus, neutrophil recruitment into the lung can be dependent on or independent of β_2 integrins (CD18) (31). β_2 integrins include Lymphocyte Function Antigen (LFA-1, $\alpha_L\beta_2$, CD11a/CD18), (Mac-1, $\alpha_M\beta_2$, CD11b/CD18), p150,95 (CD11c/CD18), and $\alpha_d\beta_2$ integrin (CD11d/CD18) (42). The CD18-dependent neutrophil migration pathway uses endothelial ICAM-1 as a counter-ligand. Both Mac-1 (43) and LFA-1 (44) appear to be very important in neutrophil recruitment into the lung in models of LPS-induced pulmonary inflammation.

3. PLATELETS

Platelets play a central role in hemostasis, wound healing, and inflammation. They originate from bone marrow megakaryocytes and contain glycogen, mitochondria, and at least three types of granules (dense core granules, lysosomes, and α -granules). The granules contain adhesion molecules, factors relevant for coagulation and fibrinolysis, calcium, and pyrophosphate (45). Platelets circulating in the blood are in a quiescent state. Following activation, platelets can secrete the content of the granules, change their shape, and up-regulate the

expression of adhesion molecules including P-selectin, PECAM-1 (CD31), glycoprotein (GP) IIb/IIIa ($\alpha_{IIb}\beta_3$) integrin, fibronectin, and thrombospondin (46).

3.1. Platelet-leukocyte-endothelium interaction

Platelets can adhere to von-Willebrand Factor (vWF) expressed on activated endothelial cells or in the subendothelial space (47). This interaction is mediated by the glycoprotein (GP)Ib/IX/V complex on platelets. This complex is constitutively expressed on platelets and consists of four gene products (GPIb α , GPIb β , GPIX, and GPV). Binding of the GPIb/IX/V complex to vWF initiates the activation of the integrin $\alpha_{IIb}\beta_3$ on platelets via “outside-in” signaling (48-51), resulting in contraction, shape change, spreading, secretion and aggregation (52-54). GPIb α is also a low affinity-ligand for P-selectin (55). Since the density of GPIb α on the platelet membrane is very high, GPIb α can mediate P-selectin-dependent rolling on activated endothelium (55) and platelet-platelet-interactions (56).

GPIIb/IIIa ($\alpha_{IIb}\beta_3$ integrin) is an important adhesion molecule on platelets, responsible for mediating platelet aggregation and some platelet-neutrophil-interactions. In the resting state, $\alpha_{IIb}\beta_3$ is able to bind immobilized but not soluble fibrin (ogen) (57). Outside-in signaling through G-protein coupled receptors leads to conformational changes of $\alpha_{IIb}\beta_3$ and subsequent binding of ligands including fibronectin, fibrinogen, vitronectin, vWF, and thrombospondin (TSP)-1 (58). Like other integrins, $\alpha_{IIb}\beta_3$ is also able to mediate “outside-in” signaling and regulate activation of platelets. Following the initial contact between platelets and endothelium and activation of the integrin, it can bind soluble vWF, fibrinogen, and other ligands. Another integrin on platelets, $\alpha_5\beta_1$, can bind fibronectin and induce stable adhesion (59). Under low shear conditions, integrins alone can mediate platelet adhesion (60), but GPIb/IX/V interaction with vWF is required for adhesion under high shear.

P-selectin, a type I membrane protein, is stored in α -granules of platelets and in Weibel-Palade bodies of endothelial cells and is rapidly brought to the cell surface following activation. P-selectin mediates the initial binding (‘capturing’) of platelets to leukocytes and leukocytes to endothelial cells. Platelet P-selectin can bind to P-selectin glycoprotein ligand (PSGL)-1 (61-63) and perhaps a second ligand (64) on different subsets of leukocytes. Following the initial capturing, firm neutrophil-platelet adhesion is mediated by binding of the leukocyte integrin $\alpha_M\beta_2$ (CD11b, Mac-1) (65, 66) to GPIb on platelets (67). In addition, fibrinogen bound to platelet $\alpha_v\beta_3$ or $\alpha_{IIb}\beta_3$ can form a bridge to leukocyte Mac-1 (68). Alternatively, Intercellular Adhesion Molecule (ICAM)-2 on platelets and $\alpha_L\beta_2$ on neutrophils can induce firm adhesion (69).

Following the adhesion of neutrophils to platelets, neutrophils are activated through PSGL-1 (62, 70, 71), the Triggering Receptor Expressed on Myeloid cells (TREM)-1 (72), lipid mediators and chemokines presented by platelets (68, 73), and integrin mediated “outside-in” signaling (74). Platelet depletion reduces neutrophil rolling and adhesion in various animal models of inflammation (75, 76).

3.2. Platelets in ALI

Platelets are involved in different inflammatory lung diseases (77, 78). Growing evidence suggests that platelets also play a crucial role in the pathogenesis of acute lung injury (79-81), but the exact mechanisms by which platelets contribute to tissue damage and influence neutrophil recruitment are still unknown.

Intrapulmonary causes of ALI, like acid aspiration and pneumonia, activate the lung microvascular endothelium, and this consecutively leads to up-regulation of adhesion molecules including ICAM-1 (44), release and presentation of chemokines (82) and lipid mediators (79). Systemic inflammatory stimuli can also induce an up-regulation of adhesion molecules on lung microvascular endothelium (80) and mediate neutrophil accumulation (83) that requires TLR4. During endotoxemia, platelets roll along and firmly adhere to lung capillary endothelial cells as shown by intravital microscopy (81). This interaction is mainly mediated by platelet P-selectin and an unknown counter-receptor on the endothelium (81). Platelets that adhere to the endothelium can become fully activated by G-protein coupled receptors (84) and integrin outside-in signaling (85), subsequently releasing chemokines and lipid mediators (79). These can either activate attached leukocytes or induce further activation of endothelial cells by releasing pro-inflammatory mediators (79). Platelet activation is associated with an up-regulation of P-selectin expression (84), which mediates ‘secondary capturing’ of neutrophils to the vessel wall. ‘Secondary capture’ is the interaction of a freely flowing leukocyte with an adherent leukocyte or platelet, leading to subsequent attachment to the endothelium and rolling. Elimination or blockade of P-selectin reduces neutrophil recruitment into the lung (79) and rescues mice from acid-induced ALI.

Although the interaction between platelets and neutrophils is mediated by several molecules, it is sufficient to block or eliminate platelet P-selectin to reduce the number of platelet-neutrophil aggregates during ALI (79). The physical interaction between these two cell types (Figure 2) leads to a reciprocal activation by activating G-protein-coupled receptors and integrin outside-in signaling (74). Due to the mechanical retention of circulating neutrophils in the narrow segments of the alveolar capillaries, it might be possible that integrin activation and binding to their ligands is sufficient for neutrophil recruitment. The pro-inflammatory mediator, thromboxane A₂ (TXA₂) was identified as an important mediator released by platelet-neutrophil aggregates in ALI (79). TXA₂ acts on endothelial cells and induces the expression of adhesion molecules, polymerization of actin, and contraction (79). However, it is likely that other lipid mediators and chemokines released from activated platelets contribute to ALI.

4. CONCLUSIONS AND FURTHER DIRECTIONS

In summary, these findings from animal studies indicate that platelets may be major pathogenic contributors in inflammatory lung diseases and ALI induced by sepsis, aspiration of gastric content, pneumonia, or ventilator-

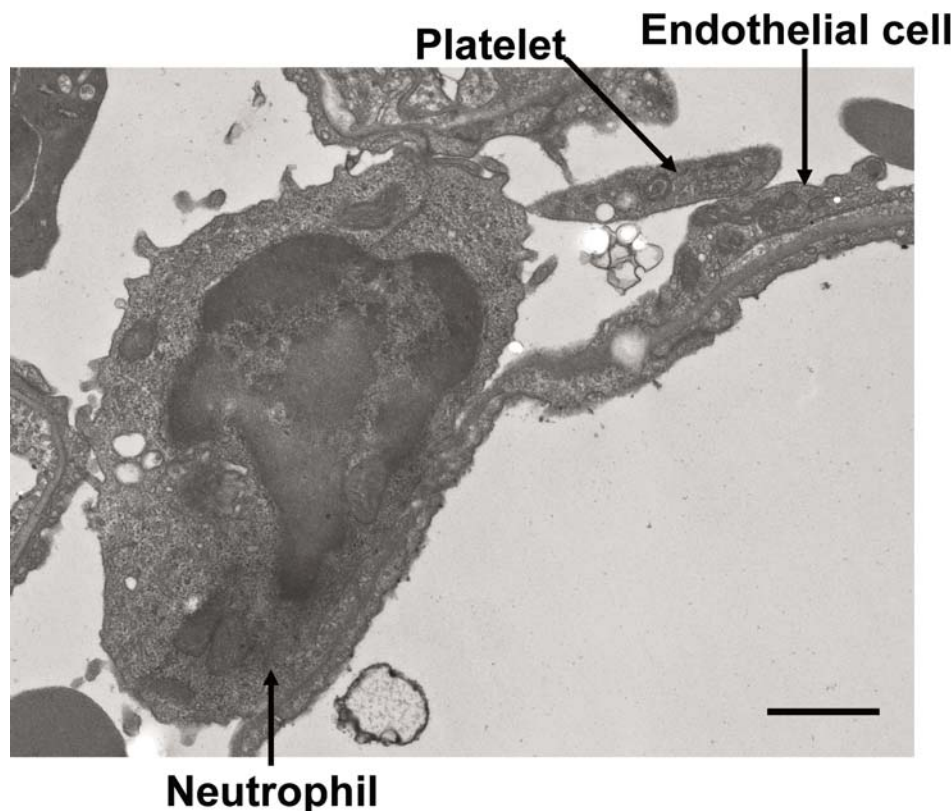


Figure 2. Platelet-neutrophil aggregate in the pulmonary microcirculation. Platelet-neutrophil interaction 30 minutes after initiation of acid-induced ALI in pulmonary microvasculature visualized by electron microscopy. Platelet attached directly to the endothelium and a neutrophil. Scale bar: 1 μ m.

induced lung injury. The precise mechanisms of the role of platelets for neutrophil recruitment into the inflamed lung and the release of pro-inflammatory mediators in ALI remain to be elucidated. Inhibition of platelets and/or platelet-neutrophil-interactions could present a very powerful and promising target in the treatment of ALI. However, further clinical trials must distinguish between infectious and non-infectious ALI, because neutrophils play a critical role in host defense. While inhibition of neutrophil recruitment may be beneficial in the abacterial phase of ALI (86), such as in aspiration-induced ALI, it could be devastating in patients with bacterial ALI.

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Abbreviations: ALI, acute lung injury; MCP-1, Monocyte Chemotactic Protein-1; cPLA2, cytosolic Phospholipase A2; TF, Tissue Factor; PAR, Protease-Activated Receptors; EPCR, Endothelial Protein C Receptor; fMLP, N-Formyl-L-Methyl-L-Leucyl-L-Phenylalanine; PECAM-1, Platelet

Endothelial Cell Adhesion Molecule-1; LFA-1, Lymphocyte Function Antigen-1; Mac-1, Macrophage antigen-1; ICAM, Intercellular Adhesion Molecule; LPS, Lipopolysaccharide; GP, glycoprotein; vWF, von-Willebrand Factor; TSP-1, Thrombospondin; PSGL-1, P-Selectin Glycoprotein Ligand; TREM-1, Triggering Receptor Expressed on Myeloid cells-1; TXA₂, thromboxane A₂.

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